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DOCUMENTS

OF THE

ASSEMBLY

OF THE

STATE OF NEW YORK

ONE HUNDRED AND FORTY-FIRST SESSION

1918

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VOL. XIV.—PART 1.—NO. 26.—PART 5

ALBANY
J. B. LYON COMPANY, PRINTERS
1918

State of New York — Department of Agriculture.

THIRTY-SIXTH ANNUAL REPORT

OF THE

New York
Agricultural Experiment Station.

(GENEVA, ONTARIO COUNTY)

FOR THE YEAR 1917.

With Reports of Director and Other Officers.

TRANSMITTED TO THE LEGISLATURE JANUARY 15, 1918.

ALBANY
J. B. LYON COMPANY, PRINTERS
1918

STATE OF NEW YORK

No. 26.

IN ASSEMBLY.

JANUARY 15, 1918.

THIRTY-SIXTH ANNUAL REPORT

OF THE

BOARD OF CONTROL OF THE NEW YORK AGRICULTURAL EXPERIMENT STATION.

STATE OF NEW YORK:

DEPARTMENT OF AGRICULTURE,

ALBANY, *January* 15, 1918.

To the Legislature of the State of New York:

As Commissioner of Agriculture, and as President of the Board of Control, I have the honor to submit herewith the Thirty-sixth Annual Report of the Director of the New York Agricultural Experiment Station, at Geneva, N. Y., in pursuance of the provisions of the Agricultural Law.

I am, respectfully yours,
CHARLES S. WILSON,
Commissioner.

NEW YORK AGRICULTURAL EXPERIMENT STATION.

W. H. JORDAN, *Director.*

GENEVA, N. Y., *January* 15, 1918.

HON. CHARLES S. WILSON, *Commissioner of Agriculture, Albany, N. Y.:*

DEAR SIR: I have the honor to transmit herewith the report of the Director of the New York Agricultural Experiment Station for the year 1917.

Yours respectfully,

W. H. JORDAN,

Director.

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5 June '31 Alb. 9. 1918 v. 2. 14: cont. 9 June '31 N. Y.

BOARD OF CONTROL.

Governor Charles S. Whitman, Albany.
Commissioner Charles S. Wilson, Albany.
Parker Corning, Albany.
Frank M. Bradley, Barkers.
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Commissioner Charles S. Wilson, *President.*
William O'Hanlon, *Secretary and Treasurer.*

STATION STAFF.

Whitman H. Jordan, Sc.D., LL.D., *Director.*

George W. Churchill, *Agriculturist and Superintendent of Labor.*

[1] Joseph F. Barker, M.S.,
[2] Reginald C. Collison, M.S., *Agronomists.*

[3] James E. Mensching, M.S., *Associate Chemist (Agronomy).*

[4] William W. Baer, B.S.,
[5] Lloyd A. Bosworth, M.S., *Assistant Chemist (Soils).*

Everett P. Reed, B.S.A., *Assistant Agronomist.*

William P. Wheeler, *First Assistant (Animal Industry).*

Robert S. Breed, Ph.D., *Bacteriologist.*

Harold J. Conn, Ph.D., *Associate Bacteriologist.*

Godfrey L. A. Ruehle, M.S.,
[6] James D. Brew, B.S.,
[7] John Bright, M.S., *Assistant Bacteriologists.*

Fred C. Stewart, M.S., *Botanist.*

Walter O. Gloyer, M.A., *Associate Botanist.*

Mancel T. Munn, M.S., *Assistant Botanist.*

Lucius L. Van Slyke, Ph.D., *Chemist.*

[8] Rudolph J. Anderson, B.S.,
Arthur W. Clark, B.S.,
John C. Baker, Ph.D., *Associate Chemists.*

Morgan P. Sweeney, A.M.,
Otto McCreary, B.S.,
Richard F. Keeler, A.B.,
William F. Walsh, B.S.,
[9] Walter L. Kulp, M.S.,
[10] Arthur J. Flume, B.S., *Assistant Chemists.*

George A. Smith, *Dairy Expert.*

Frank H. Hall, B.S., *Vice-Director; Editor and Librarian.*

Percival J. Parrott, M.A., *Entomologist.*

Hugh Glasgow, Ph.D.,
[11] Fred Z. Hartzell, M.A., *Associate Entomologists.*

Harold E. Hodgkiss, B.S.,
Bentley B. Fulton, M.S., *Assistant Entomologists.*

Ulysses P. Hedrick, Sc.D., *Horticulturist.*

Roy D. Anthony, M.S.A.,
[11] Fred E. Gladwin, B.S., *Associate Horticulturists.*

George H. Howe, B.S.A.,
Joseph W. Wellington, B.S.,
[12] William C. Stone, M.S., *Assistant Horticulturists.*

Orrin M. Taylor, *Foreman in Horticulture.*

F. Atwood Sirrine, M.S., *Special Agent* (Riverhead, N. Y.).

Jessie A. Sperry, *Director's Secretary.*

Frank E. Newton,
Willard F. Patchin,
Lena G. Curtis,
[13] Agnes Ryan,
Maude L. Hogan,
[14] Emily L. Drodesky,
[15] K. Loraine Horton, *Clerks and Stenographers.*

Elizabeth Jones, *Computer and Mailing Clerk.*

Address all correspondence, not to individual members of the staff, but to the New York Agricultural Experiment Station, Geneva, N. Y.

The Bulletins published by the Station will be sent free to any farmer applying for them.

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[1] Resigned Sept. 1, 1917.
[2] Appointed Sept. 1, 1917; previously Associate Chemist (Agronomy).
[3] Appointed Sept. 1, 1917.
[4] Entered Naval Service of the United States, July 1, 1917.
[5] Appointed July 30, 1917; resigned Sept. 1, 1917.
[6] Resigned Dec. 31, 1917.
[7] Appointed Jan. 1, 1918.
[8] Entered Military Service of the United States, Dec. 1, 1917.
[9] Appointed May 21, 1917.
[10] Resigned May 20, 1917.
[11] Connected with Grape Culture Investigations, Fredonia, N. Y.
[12] Appointed Jan. 1, 1917.
[13] Resigned April 30, 1917.
[14] Appointed May 7, 1917; resigned Oct. 11, 1917.
[15] Appointed Nov. 22, 1917.

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THIRTY-SIXTH ANNUAL REPORT

OF THE

Board of Control of the New York Agricultural Experiment Station.

TREASURER'S REPORT.

GENEVA, N. Y., *July* 1, 1917.

To the Board of Control of the New York Agricultural Experiment Station:

As Treasurer of the Board of Control, I respectfully submit the following report for the fiscal year ending June 30, 1917:

APPROPRIATIONS, 1915–1916.

INVESTIGATIONS.

1916	*Receipts.*	*Dr.*
July 1.	To balance on hand	$3,835 66

Expenditures.	*Cr.*
By office equipment	$40 50
By maintenance	5 52
By laboratory equipment	2,760 59
By laboratory maintenance	40 95
By farm equipment	6 00
By farm maintenance	278 61
By traveling expenses	20 36
By miscellaneous	51 38
By remitted to State Treasurer	629 42
By balance	2 33
	$3,835 66

GENERAL EXPENSES — HEAT, LIGHT, WATER, ETC.

1916.	*Receipts.*	*Dr.*
July 1.	To balance	$919 73
	To amount received from Comptroller	932 08
		$1,851 81

Expenditures.	*Cr.*
By real estate maintenance	$30 25
By heat, light and water	1,818 49
By balance	3 07
	$1,851 81

1916.	SALARIES.	*Dr.*
July 1.	To balance	$5,097 34

Expenditures.	*Cr.*
By remitted to Treasurer	$453 41
By balance	4,643 93
	$5,097 34

LABOR.

1916.		Dr.
July 1.	To balance	$2,219 94

	Expenditures.	*Cr.*
	By remitted to Treasurer	$1,448 19
	By balance	771 75
		$2,219 94

GRAPE INVESTIGATION.

1916.		Dr.
July 1.	To balance	$714 02

	Expenditures.	*Cr.*
	By laboratory equipment	$6 85
	By laboratory maintenance	3 20
	By farm maintenance	1 93
	By remitted to State Treasurer	702 04
		$714 02

FERTILIZERS, FEEDING STUFFS, ETC.

1916.	*Receipts.*	*Dr.*
July 1.	To balance	$608 25
	To amount received from Comptroller	281 17
		$889 42

	Expenditures.	*Cr.*
	By laboratory equipment	$575 93
	By laboratory maintenance	66 44
	By traveling expenses	5 87
	By remitted to State Treasurer	241 18
		$889 42

FIELD, ORCHARD AND MILK INVESTIGATIONS.

1916.	*Receipts.*	*Dr.*
July 1.	To balance	$531 19

	Expenditures.	*Cr.*
	By remitted to Treasurer	$531 19

APPROPRIATIONS, 1916–1917.

MAINTENANCE AND OPERATION.

1916.	*Receipts.*	*Dr.*
July 1.	To amount received from Comptroller	$28,381 85

Expenditures.	*Cr.*
By fuel, light and water	$2,197 60
By printing	375 80
By equipment	5,338 42
By supplies	8,727 95
By hired horses and vehicles	2,495 01
By traveling expenses	3,361 74
By communications	1,661 65
By general plant service	499 97
By rent	993 65
By motor truck	625 00
By remitted to Treasurer	166 27
By balance	1,938 79
	$28,381 85

RESEARCH GENERAL.

1916.	*Receipts.*	*Dr.*
July 1.	To amount received from Comptroller	$41,424 56

Expenditures.	*Cr.*
By salaries, regular	$41,357 81
By remitted to Treasurer	66 75
	$41,424 56

ADMINISTRATION.

1916.	*Receipts.*	*Dr.*
July 1.	To amount received from Comptroller	$12,866 60

Expenditures.	*Cr.*
By salaries, regular	$12,808 29
By remitted to Treasurer	58 31
	$12,866 60

INVESTIGATIONS, LABOR.

1916.	*Receipts.*	*Dr.*
July 1.	To amount received from Comptroller	$17,232 87

Expenditures.	*Cr.*
By wages, temporary	$2,072 12
By wages, regular	15,160 74
By remitted to Treasurer	01
	$17,232 87

RESEARCH: GRAPE CULTURE.

1916.	*Receipts.*	*Dr.*
July 1.	To received from Comptroller	$3,799 93

Expenditures.	*Cr.*
By salaries, regular	$3,799 92
By remitted to Treasurer	01
	$3,799 93

GRAPE INVESTIGATION: LABOR.

1916.	*Receipts.*	*Dr.*
July 1.	To received from Comptroller	$892 00

Expenditures.	*Cr.*
By wages, regular	$312 00
By wages, temporary	579 10
By remitted to Treasurer	90
	$892 00

GRAPE INVESTIGATION: SPECIAL SERVICE.

1916.	*Receipts.*	*Dr.*
July 1.	To received from Comptroller	$104 13

	Expenditures.	*Cr.*
	By salaries, temporary	$75 00
	By remitted to Treasurer	29 13
		$104 13

FIELD, ORCHARD AND MILK INVESTIGATION: RESEARCH AND LABOR.

1916.	*Receipts.*	*Dr.*
July 1.	To received from Comptroller	$13,276 94

	Expenditures.	*Cr.*
	By salaries, regular	$10,149 72
	By wages, regular	2,298 40
	By wages, temporary	828 68
	By remitted to Treasurer	14
		$13,276 94

INSPECTION OF FERTILIZERS, FEEDING STUFFS, ETC.: SALARY AND LABOR.

1916.	*Receipts.*	*Dr.*
July 1.	To received from Comptroller	$14,370 49

	Expenditures.	*Cr.*
	By salaries, regular	$12,937 71
	By wages, regular	1,165 40
	By wages, temporary	263 42
	By remitted to Treasurer	3 96
		$14,370 49

GENERAL PLANT SERVICE.

1916.	*Receipts.*	*Dr.*
July 1.	To received from Comptroller	$801 50

	Expenditures.	*Cr.*
	By general plant service	$801 50

INSPECTION FERTILIZER, FEEDING STUFFS, ETC.: MAINTENANCE AND OPERATION.

1916.	*Receipts.*	*Dr.*
July 1.	To received from Comptroller	$1,734 10

	Expenditures.	*Cr.*
	By fuel, light, power and water	$390 04
	By equipment	128 43
	By supplies	630 84
	By traveling expenses	143 84
	By communications	112 34
	By remitted to Treasurer	112 91
	By balance	215 70
		$1,734 10

APPROPRIATIONS, FEDERAL GOVERNMENT.

HATCH FUND.

1916.	*Receipts.*	*Dr.*
July 1.	To received from the Treasurer of the United States as per appropriation for fiscal year ended June 30, 1917, as per act of Congress approved March 2, 1887	$1,486 97
	To balance	13 03
		$1,500 00

Expenditures.	*Cr.*
By fertilizers	$59 05
By labor	359 92
By traveling expenses	209 88
By salaries	849 92
By balance	21 23
	$1,500 00

ADAMS FUND.

1916.	*Receipts.*	*Dr.*
July 1.	To received from the Treasurer of the United States as per appropriation for fiscal year ended June 30, 1917, as per act of Congress approved March 2, 1887	$1,365 04
	To balance	134 96
		$1,500 00

Expenditures.	*Cr.*
By salaries	$1,499 84
By balance	16
	$1,500 00

RING MEMORIAL FUND.

Receipts.	*Dr.*
To balance on hand July 1, 1917	$1,000 00

I have received and remitted to the State Treasurer for the fiscal year ending July 1, 1917, for produce sold	$3,470 66

All expenditures are supported by vouchers approved by the Auditing Committee of the Board of Control and have been forwarded to the Comptroller of the State of New York.

(Signed) WILLIAM O'HANLON,
Treasurer.

DIRECTOR'S REPORT FOR 1917.*

To the Honorable Board of Control of the New York Agricultural Experiment Station:

Gentlemen.— I have the honor to present to you a report of the operations of this institution, for the calendar year 1917, together with a review of changes that have come to the Station during the year, and a statement of certain of its needs.

It cannot be said at the end of any short period of time that the general activities of the Station have materially changed. It is true that new problems arise and old ones are set aside. These problems always relate to agricultural production, and the use of crops in the form of cattle foods and human foods. Much of the effort of the Station is directed toward defense of farmers and fruit growers against the devastation of insect and fungus pests. It is also our duty to assist the Commissioner of Agriculture in the inspection of such commodities as fertilizers and cattle feeds, and fungicide and insecticide materials.

The institution has not escaped the effects of the war. Not only have members of the Station Staff been called into service, but throughout the entire institution there is felt the depressing influence of the gigantic and critical conditions in which this nation is engaged. All communities and institutions now are in the midst of an atmosphere unfavorable to the concentration of thought and effort on the usual activities of life.

ADMINISTRATION.

STATION STAFF.

No year passes without more or less change in the personnel of the Station Staff.

Joseph F. Barker, M.S., who was connected with the institution as Agronomist since 1911, resigned his position on September first. The occasion of his resignation was a call to a responsible position in the Ohio State University at an increased salary and to opportunities which he regarded as especially desirable. During the time Mr. Barker was connected with the Institution he gave to his work industry and zeal, his earnest effort being to accomplish for the agricultural interests of the State the greatest amount of good.

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* This is a reprint of Bulletin No. 445, December, 1917.

Lloyd A. Bosworth, M.S., who occupied the position of Assistant Chemist for the brief space of one month resigned his position to accept a position in the Philippine Islands.

Reginald C. Collison, M.S., Associate Chemist, because of the satisfactory services which he had rendered the Institution since his appointment in 1912, was promoted to the position vacated by Mr. Barker, his appointment taking effect on September 1, 1917.

James E. Mensching, M.S., was appointed Associate Chemist to fill the vacancy created by the promotion of Mr. Collison. His term of service began on September first. Mr. Mensching is a graduate of the Ohio State University and at the time of his appointment was associated with Dr. H. P. Armsby in Animal Nutrition work at the Pennsylvania Experiment Station.

Walter L. Kulp, M.S., was appointed in the position of Assistant Chemist to fill the place of Arthur J. Flume, his appointment taking effect on May 20, 1917.

Rudolph J. Anderson, B.S., Associate Chemist, having offered his services to the Federal Government, has been assigned to a position in the Surgeon General's Department with the rank of Captain. Mr. Anderson's work will be related to the feeding of the army, a service which he is admirably fitted to render.

William W. Baer, B.S., Assistant Chemist, enlisted in the United States Navy and was called into service in June. After the necessary training he has been given the rank of Ensign.

Donald B. Clayton, being a member of Company B, of the National Guard was called into service very soon after the declaration of war and is now serving as bugler in an officers' corps.

F. H. Hall, B.S., Vice-Director, Editor and Librarian, has been requisitioned by the Federal Food Administration to aid in preparing various publications and utterances sent out from that division of our Federal Government. His experience for nearly twenty years as Editor of the publications of this Institution, a service requiring a broad knowledge of the various phases of agricultural science, has admirably fitted Mr. Hall for the duties he is now fulfilling. It is understood that he is rendering highly satisfactory services, while it is still possible for him to carry on most of the editorial work of the Station.

The Director of the Station very soon after the declaration of war began to interest himself in food conservation and has endeavored through public addresses and the instruction of classes in war dietetics to promote such economy in the use of food resources as is required by war conditions. Later he was asked by Mr. Hoover to serve on the Federal Milk Commission for the purpose of establishing milk prices for the City of New York. This service is not yet completed.

MAINTENANCE FUND.

The following appropriations were made available for the use of the Station during the fiscal year beginning July 1, 1917.

GENERAL FUND

Salaries		
Administration	$13,260 00	
Research	56,400 00	$69,660 00
Labor		
Wages, regular	$6,760 00	
Wages, temporary	15,200 00	21,960 00
		$91,620 00
Maintenance and operation		31,830 00
		$123,450 00

INSPECTION FUND.

Salaries	$13,530 00
Labor, regular and temporary	1,515 00
Total for inspection	$15,045 00
Total appropriation	$138,495 00
Salaries for all divisions	$83,190 00
Labor, regular	7,600 00
Labor, temporary	15,875 00
Maintenance and operation	31,830 00
	$138,495 00

The amount appropriated was $6,379.80 less than the request of the Board of Control.

In accordance with the action of your Board the following budget for the fiscal year beginning July 1, 1918, has been presented to the

Governor's Budget Committee. It is not yet acted upon by the Legislative Budget Committee.

GENERAL FUND.

Salaries		
Administration	$13,560 00	
Research	59,250 00	
		$72,810 00
Labor		
Wages, regular	$7,020 00	
Wages, temporary	20,000 00	
		27,020 00
		$99,830 00
Maintenance and operation		63,075 00
		$162,905 00

INSPECTION FUND.

Salaries	$13,830 00
Labor, regular and temporary	1,600 00
Total for inspection	$15,430 00
Total appropriation	$178,335 00

Salaries for all divisions	$86,640 00
Labor, regular	7,920 00
Labor, temporary	20,700 00
Maintenance and operation	63,075 00
	$178,335 00

In addition to the above for salaries, labor, maintence and operation, the budget contains requests for the following:

Repairs	$3,150 00	
New construction*	70,000 00	
		73,150 00
Total requested		$251,485 00

---

* Remainder of $100,000 appropriated, but not made immediately available for administration, demonstration and library building.

LABOR SITUATION.

It is to be noted that the amount asked for the maintenance of labor at the Station is considerably increased over that received during the present fiscal year. This increase is necessary because of the fact that it is not possible to obtain efficient labor at the wages now paid. The Institution is unfortunate in being located very near a munition plant where wages are rated at an abnormally high figure. It is, therefore, very difficult for the Station to secure labor at the wages that would ordinarily be paid. This fact, taken in connection with the general scarcity of labor, makes the situation very difficult. During the past season it has not been possible to obtain desirable labor at any price which could be paid within the resources of the Institution.

SALARY CONDITIONS.

It is universally true that when for any reason living costs materially increase, persons occupying salaried positions are placed at a disadvantage because increases in salaries, if realized at all, come very much later than the increased living expenses. It is for this reason that the members of the Station Staff receiving the smaller salaries are feeling very seriously the effect of the greater cost of rent, clothing and food supplies. It seems unfortunate that while the needs of organized labor in this particular are met quite promptly, teachers, scientific workers, clerks and similar employees receive at first scant consideration.

NEW BUILDING.

The Legislature of 1915 appropriated $100,000.00 for the construction and equipment of an Administration, Demonstration and Library building for this Institution. Of this appropriation $30,000 was made immediately available, under which construction began; and the building is now roofed in and is ready for interior construction. Varying conditions, including scarcity of labor and the slowness with which materials have been secured and transported have seriously delayed work on this structure. It now appears that it will probably be ready for occupancy some time during the summer of 1918.

FURTHER BUILDING NEEDS.

In previous reports attention has been called to the fact that new plant houses and a cold storage house must be soon erected at the Institution if it is to continue present lines of work. The report presented to you for 1916 contained the following statement:

" The time has come when in order to carry on its work with the desired efficiency new and greatly enlarged plant houses should be provided. The present plant houses of the Station were erected about twenty-five years ago. They have exceeded the usual life of such structures, and are now neither adequate nor efficient. There is a large amount of work in agricultural investigation which should be carried on in such houses, if carried on at all, including plant breeding, plant nutrition and studies of plant diseases and injurious insects, to all of which lines the Station is obliged to give much attention.

"The small cold-storage house, established chiefly for the storing of fruits, was erected at the Station something more than twenty years ago. The preservation of fruits, of which the Station has several thousand varieties, requires cold storage facilities in order that such materials may be used for study and exhibition purposes. The present cold-storage plant is inadequate in size and construction and if retained will need enlargement and extensive repairs. A new building should be erected.

"It is not to be expected that an institution which has been in operation for thirty-four years should not find it necessary to replace buildings of a somewhat perishable character that have been in use during that time. The State must either provide the means for such replacement or allow the institution to deteriorate seriously in appliances and efficiency."

RELATION OF THE STATION TO WAR CONDITIONS.

It is an encouraging fact that the institutions established through Federal and State aid, without exception, have patriotically been held ready for such services as it has been possible for them to render in accomplishing the purpose for which this Nation entered the war. If there ever has been any serious question as to the wisdom of establishing the Colleges of Agriculture and the Agricultural Experiment Stations, all doubts of this kind should certainly now disappear. The war in which we are now engaged is one which is testing all the resources of the Nation, particularly our food supply. Because we must not only feed ourselves but the Allies who have been facing the enemy so long and whose productive capacity has been greatly diminished through the entering of forty or more million men into war service, it is incumbent upon us to increase the production of food stuffs if possible and to conserve to the fullest extent that which we produce. It is self-evident, therefore, that the knowledge which has been accumulated through agricultural research and which has been disseminated through the agricultural colleges and experiment stations is now of the highest importance. Our present knowledge of the means of increasing crop production and of defending farmers against the inroads of pests is serving us at a critical time and without such knowledge our condition would be much more serious than it is. More than this, a large number of men trained in our colleges of agriculture are rendering war service of the highest importance. This Nation will sometime learn, as we trust it is now learning, that its efficiency depends, and always will depend, upon our understanding of how to develop and conserve our material resources as well as upon the expert service which trained men may render in agriculture and our industrial life.

The withdrawal of so many men from the faculties of the colleges of agriculture and the staffs of the experiment stations has more or less depressed the activities of these institutions. The situation has also caused men whose ordinary function is that of research or teaching to engage more largely in the efforts of popular education. No criticism should be offered concerning this condition, because the first duty of every man now is to aid the Nation at the most critical points in bringing the war to a successful conclusion.

There has been much inquiry and discussion concerning the activities of experiment stations as a means of rendering special services under war conditions. It does not seem that there are many new efforts which could be undertaken to advantage. The work of these institutions has been directly in the line of increasing our knowledge of the factors which enter into crop production and concerning the means for defending crops of various kinds against pests. It would seem, therefore, that the only course to pursue is to continue these investigations, with occasional deviations of effort to meet special war conditions. What we have been doing, however, would seem to be what we should continue to do in the most earnest and energetic manner possible.

STATION PUBLICATIONS.

The distribution of Station publications for the year has been in accordance with the following figures:

POPULAR BULLETINS

Residents of New York	38,859
Residents of other States	2,526
Newspapers	778
Experiment Stations and their staffs	2,295
Miscellaneous	100
Total	44,558

COMPLETE BULLETINS.

Experiment Stations and their staffs	2,526
Libraries, scientists, etc	400
Foreign list	330
Individuals	4,086
Miscellaneous	100
Total	7,442

To the list of bulletins as given on page 31 two other publications should be added as distributed during 1917 — the Thirty-fourth Annual Report for 1915, and Part II of the Annual Report for 1916. The usual delay in printing of the Annual Reports of the Station has been intensified during the past year so that distribution of the report for 1915 could not be made for a year and a half after the completion of the year. For the first time, also, printing and delivery of the bulletins of the Station have been so delayed as to be annoying and embarrassing. Many factors have combined to cause this delay, including failure of the Legislature to make the necessary appropriation for printing the bulletins, difficulty in securing paper of proper quality, and slow transmission of printed material from Albany to Geneva because of transportation troubles. That the bulletins have appeared at all during the last half of 1917 has been due to the public spirit of the State Printer, the J. B. Lyon Company, who bore the financial burden of their publication in advance of an appropriation to provide for them.

Part II of the report for 1916, mentioned above, is The Peaches of New York, the fifth of the series of fruit monographs prepared by the Horticultural Department of the Station. This volume has been more in demand than any of its predecessors except The Apples of New York, since it is one of the most satisfactory and pleasing of the series. The Commissioner of Agriculture very kindly placed at the disposal of the Station a portion of his allotment of 2000 copies, thus aiding in meeting the great demand, which our own allotment of 2000 copies could not possibly cover. The remaining 5000 copies of the monograph have been assigned to the members of the Legislature. Attention must again be called to this unsatisfactory method of distribution, whereby it is made impossible to place such valuable books in the hands of fruit growers who would make profitable use of them, while hundreds of copies go to ornament the libraries of those who have no connection with fruit production and are not specially interested in it, but desire the books because of their beautiful color plates and because they can be obtained without expense.

RESULTS OF STATION WORK IN 1917.

During 1917, the published results of Station activities appeared in seventeen bulletins, of which nine had popular editions also; in seven technical bulletins; and in four circulars. The number of persons reached by these Station publications increased somewhat during the year, but there are evidently still thousands of farmers in the State who might benefit by Station experience who secure knowledge of its results only at second or third hand, if at all.

DIVISION OF AGRONOMY.

Ground limestone for New York State.— The need for lime in New York agriculture has been shown in many ways and the production of ground limestone in the State has developed very rapidly in five years to meet this need, at least in part. From one plant grinding limestone in 1912 and one producing marl, the number has increased until there were 56 such plants in the State in 1917 and a dozen or more outside the State shipping in large quantities of the ground stone. To familiarize farmers with these local sources of supply and the comparative value of the product of the different plants, Bulletin No. 430 was prepared. This includes a map showing the location of the different grinding plants and a table giving the analysis of the ground product of each, the analyses being more detailed as well as more numerous than those given in the fertilizer bulletin. Discussions are also presented, in simple terms, of the comparative value of ground limestone, of the degree of fineness required for best results, of soils requiring lime, of field tests for need of lime, of the response of crops to liming and of the nature of soil acidity.

Determination of carbonates.— Technical Bulletin No. 62 describes and illustrates a simple piece of apparatus, devised by the Agronomist, for the determination of carbonates, particularly in limestone and similar materials. It is based on the principle of the hydrometer, requires no weighing and gives results without computations. From its inexpensiveness, simplicity and accuracy it should serve a useful purpose in a wide field.

Lysimeter and pot-culture work.— During 1914 the Station installed a battery of lysimeters for soil study, perhaps the most complete and expensive piece of apparatus of this type in the country. Believing that familiarity with this lysimeter installation would be of value to others engaged in similar work or contemplating it, plans, photographs and structural details of the apparatus have been collected in Technical Bulletin No. 61. Outlines of the problems to be studied and of the plan of work are also given. Adjacent to this battery of lysimeters on the Station grounds are sets of drain-tile cylinders for controlling conditions in certain lines of soil study, but without opportunity to study drainage water as given by the lysimeters. The two installations supplement each other.

FIELD WORK OUTSIDE STATION FARM.

Apple orchard — Fertilization, cultivation and cover crop tests..................	Great Bear Springs Co. (8 acres), Fulton. R. B. Densmore (8 acres), Albion. Jas. Vick & Sons (8 acres), Elm Grove.
Cherry orchard — Fertilization, cultivation and cover crop tests..................	P. F. O'Neil (3 acres), Geneva.
Pear orchard — Fertilization, cultivation and cover crop tests..................	Lawrence Howard (3 acres), Kinderhook.
Vineyard — Fertilization and deep plowing tests..........................	S. E. Stone, Jr. (4 acres), Fredonia. C. H. Lohman, D. W. Blood (2 acres), Dunkirk.
Tobacco culture experiment............	F. A. Tuerk (12 acres), Baldwinsville.
Four year rotation and fertilizer tests....	P. R. Bennett (6 acres), Milford.

DIVISION OF ANIMAL INDUSTRY.

Goat's milk for infant feeding.— For several years the Station maintained a herd of milch goats, the original animals coming as a gift from Mr. H. S. Greims of New York City. The animals that reached Geneva early in 1910, forty-nine in number, were of several breeds, pure and crossed; several were in poor condition; and a few later developed the somewhat uncommon malady known as takosis. Owing to these conditions and the necessity of weeding out undesirable types and animals, no records of great value were secured in 1910 or 1911; but during 1912 data were obtained showing the approximate food cost of maintaining twenty-eight females, three males and nine kids of Long-haired and Short-haired Toggenburg, Schwartzenberg, and Saanen breeds, and crosses among these breeds and of some of them with Angora and American goats. The food cost of the milk for all of the goats during 1912 was 4 cents a quart. The lowest cost for a year's production was with a Saanen doe, the estimated cost of whose milk during 1911 was 1.27 cents a quart. Even the best of these figures shows the food cost of milk from these goats to be far above that from the Station herd of Jersey cows, with which the average for three years was .92 cents a quart.

The milk of the goats was used with good success for feeding infants in a hospital. In eighteen cases where children in private homes were not thriving on other foods that had been used, a satisfactory state of nutrition was established thru the use of goat's milk, the beneficial results in some instances being very marked. With some of these children their condition was regarded as serious, and their restoration to a satisfactory state of health was good evidence that goat's milk is often a very desirable resort for infant feeding. The details of this work with milch goats appear in Bulletin No. 429.

BACTERIOLOGICAL AND DAIRY DIVISIONS.

Dairy investigations.— Definite progress has been made during the year on the studies of the accuracy of the analytical methods used for counting bacteria in milk. This has resulted in the preliminary publication in the *Journal of Dairy Science* of the results of the cooperative analytical work done with the Department of Dairy Industry of the State College of Agriculture. The chief conclusion reached in this work was that when samples of milk were prepared in such a way that all of the well known and commonly understood difficulties which prevent accurate counting of bacteria in milk were eliminated, different analysts could secure reasonably close duplicate counts. Two methods of analysis were used: one, the generally used agar plate method and the other, the direct microscopic examination of the milk previously described in bulletins from the Station. The counts secured by both methods agreed so closely that there is good reason to believe that the figures obtained really represented a very close approximation to the actual number of bacteria present.

Unfortunately, however, for the accuracy of the counts used in the milk control work in the State, the results of a long series of comparative analyses in which both the agar plate and the direct microscopic examination were used have shown that the ordinary conditions prevailing in market milk samples are such that the officially recognized counts are not counts of the individual bacteria but are counts of groups of bacteria. The results of these studies have been published as Bulletin No. 439. Since the clumps of bacteria in ordinary market milk are of variable sizes in different samples of milk, the counts secured are not only much smaller than they should be but the errors in count are so variable that it makes it uncertain whether a milk sample stated to contain 10,000 bacteria per c.c. actually contains less bacteria than does another sample stated to contain 20,000 bacteria per c.c.

These findings would leave the matter of milk control on the basis of the bacteria count in a very unfortunate condition if it were not for the fact that while these studies have been in progress still another line of experimental work has been carried out at Hobart, N. Y., and at Geneva in cooperation with dairymen and certain milk distributing companies. The report on this work published as Bulletin No. 443 shows that it is not necessary to make accurate and detailed counts of the bacteria in milk in order to control its bacterial quality; and that either the microscopic method or the officially recognized agar-plating method can be used to divide unpasteurized milk on the basis of the bacterial count into as many as three classes with an agreement in results as high as 91 per ct. Strong presumptive evidence was secured for believing that milk delivered to the milk stations by dairymen was graded more accurately by microscopic examination than by the plating method.

The results of the studies on sanitary milk problems carried out at this Station and at the Illinois, Cornell, and Wisconsin Stations have led to the publication of a joint bulletin, No. 438, which discusses the fundamental problems underlying the grading of milk according to its quality. During the year the studies upon milking machines as factors in the production of sanitary milk have been completed and the results of the investigation are being prepared for publication.

Soil flora studies.— A series of technical bulletins has been issued dealing with the general bacteriological flora of the soil. The general characteristics of the soil flora and the methods best adapted to studying it are discussed in Technical Bulletin No. 57, the first of this series. The other three bulletins of this series deal with the three groups of soil bacteria already mentioned in the Director's Report for 1916. Technical Bulletin No. 58 takes up the spore-forming bacteria, giving descriptions of four different species, three of which have been found in all the soils studied. It is shown that these are not the most abundant nor apparently the most important bacteria in soil. Technical Bulletin No. 59 deals with the non-spore-forming bacteria, showing that they are the most numerous and apparently the most active bacteria in soil, but that they are so difficult to study that it is almost impossible to identify any of them with previously described species. These organisms are considered to be so important in soil that a more intensive study of some of them and of their activity in the decomposition of organic matter in soil is now being made. The other large group of soil microorganisms, the Actinomycetes, is treated in the last paper of this series, Technical Bulletin No. 60. These organisms appear to be numerically quite important; but certain technical difficulties make it hard to tell whether or not they are very active in soil. Identification of the forms found in the soils studied has proved impossible as yet. Some of them are closely related to, if not identical with, the organism causing potato scab. The chief point emphasized by these soil flora studies is the meagerness of present-day knowledge in regard to the microorganisms of soil. One of the first steps which appears to be necessary in order to put the knowledge of soil bacteria on a scientific basis is a classification of them. The study reported in this series of bulletins is a preliminary step toward such a classification; but much work remains to be done.

Potato scab investigation.— The similarity between certain soil Actinomycetes and the organism causing potato scab has led some soil investigators to believe the cause of this disease to be widespread in soil. If it is a very common soil organism the control of the disease must be based upon different principles from those in general practice. To test out this matter an investigation has been begun this year in coöperation with the Botanical Department of the University of Illinois, but results are not yet ready for publication.

The direct microscopic examination of bacteria in soil.— A method has been worked out for examining the bacteria of soil directly under the microscope. This is quite similar to the method already used for studying milk bacteria microscopically, but the development of a satisfactory technic has proved more difficult. The method used and a few of the most interesting results obtained with it are shortly to be published as a technical bulletin.

COÖPERATIVE STUDIES.

Potato scab investigations	Department Botany, University of Illinois.
Stable and milk sanitation	Illinois Agricultural Experiment Station.
Methods of counting bacteria in milk	State College of Agriculture, Ithaca, N. Y.
Methods of controlling the sanitary quality of market milk	White Springs Farm Dairy Company. Geneva Milk Company. Geneva Board of Health, and about 40 dairymen in the vicinity of Geneva.

BOTANICAL DIVISION.

Blackheart and the aeration of potatoes in storage.— The peculiar behavior of some potato tubers in an air-tight jar led to an investigation of the effect of scant aeration upon potatoes in storage. The results of this work are given in Bulletin No. 436.

It was learned that potatoes cannot long endure close confinement. Within a certain length of time, which varies with the temperature and quantity of air available, tubers confined in hermetically sealed jars become moist over a part or the whole of their surface. If the tubers are then exposed to the air the moist surface areas turn brown and the color of the flesh at the center changes from white to pink then to black, producing a condition known as blackheart.

With a volume of air equal to the volume of the tubers a confinement of ten or twelve days is sufficient to produce the symptoms described provided the temperature is around 70° F. At 50–60° F. about twenty days are required; and at 40° F. a still longer time — somewhere between twenty-three and forty days.

Tubers confined in sealed jars with less than about ten times their volume of air are unable to do more than barely start sprouts. For normal sprouting about nineteen volumes of air per volume of tubers are required. Blackheart may be expected to appear whenever the volume of air available to the tubers is less than that required for normal sprouting.

Tubers suffering from insufficient aeration thru deep piling behave, in a general way, like tubers in sealed jars. They sprout feebly or not at all, become moist on the surface, discolor externally upon exposure to the air and are often affected with blackheart internally. While the data obtained from the experiments are insufficient for the formulation of definite rules, it appears that six feet should be the maximum depth of piling when potatoes are to be stored for several months at temperatures below 45° F. At temperatures above 50° F. the depth limit should be three feet if the potatoes are to be stored longer than three or four weeks.

Insufficient aeration during storage does not cause spindling sprout. It may retard sprouting temporarily, but when, subsequently, the tubers are supplied with air they sprout normally if at all.

Blackheart sometimes occurs in potatoes stored out-of-doors in piles and is due, undoubtedly, to insufficient aeration. It may be produced, also, by exposure to a temperature of 100–113° F. for fourteen to eighteen hours and often results from the overheating of potatoes during shipment in stove-heated cars.

The facts brought out in this investigation emphasize the importance of providing ventilation for potatoes in storage.

Neck-rot disease of onions.— During the past four years an exhaustive study has been made of a destructive rot of onions occurring in storage houses. Of the several common names by which this disease is known, neck-rot appears to be the most appropriate. Affected bulbs bear irregular, black, compact masses of fungus material (sclerotia) and a growth of smoke gray mold. These sclerotia and the mold are two different forms of the causal fungus which, tho long known, appears to be an undescribed species. Accordingly, it has been described and named *Botrytis allii.*

It has been determined that infection of the tubers takes place in the field and that the source of infection may be either wind-borne spores or fungus in the soil. A few of the infected bulbs show the disease while still in the field; but in the great majority of cases the disease does not develop until after the bulbs have been placed in storage. Also, flowers of onions grown for seed may become infected by the fungus and blast.

Factors favorable to infection and the occurrence of neck-rot are: (1) Immaturity and imperfect curing of the bulbs; (2) the application of commercial fertilizers late in the season or in incorrect proportions; (3) the application of large quantities of stable manure before planting; (4) poor air drainage on the fields; (5) high humidity, high temperature and poor ventilation in the storage house.

Methods of control appear to lie mainly along the line of field sanitation, care of the curing crop and storage in houses which are properly constructed and regulated. Diseased bulbs should not be used as " mother bulbs " for producing the seed crop, since they are an important source of infection to the seed heads and, later, to growing crop onions in nearby fields. Refuse onions should not be piled near onion fields or used for fertilizer thereon. Care should be taken to avoid unnecessary bruising, on the topping machine and while handling, of stock to be stored. The crop should mature and die early. All refuse tops, soil and screenings should be removed. The bulbs should be thoroly cured in the field or drying sheds and stored in slatted crates in well constructed houses having ample facilities for ventilation and the maintenance of low temperature.

Fumigation of the stock with formaldehyde gas either before or during storage has proven ineffective. While spraying the growing crop with bordeaux mixture has given some promising results it has not been tested sufficiently to warrant definite recommendations. Apparently, spraying is unpopular with onion growers because of certain difficulties encountered. Bulletin No. 437 gives a detailed account of these onion neck-rot studies.

ENTOMOLOGICAL DIVISION.

Plant lice injurious to apple orchards.— Bulletin No. 431 is the second report of life-history studies and experiments with the apple aphides (*Aphis sorbi, avenæ* and *pomi*), which have for their object the establishment of efficient spraying practices for the protection of bearing apple orchards. In experiments with the insects all species attacked succulent tissues, as blossom and fruit stems, tender leaves and young apples. As a result of their activities various distortions of apples developed which were, as a rule, much more conspicuous with the fruits attacked by the rosy aphis (*A. sorbi*). In an experiment on the Station grounds, that is discussed with considerable detail, an application of lime-sulphur and nicotine solution at recommended strengths afforded efficient protection from the oat aphis and the rosy aphis. Of twelve auxiliary experiments, nine gave appreciable benefits from spraying. As losses by aphides vary according to locality and season, growers who are not certain as to the necessity of systematic spraying are advised to conduct a test for a period of years, for which brief directions are given.

The radish maggot.— In Bulletin No. 442 attention is directed to the injurious work of the maggot (*Phorbia brassicæ* Bouché) on radishes and to experiments with screening as a means of protecting radish beds. Radishes produced in the spring months in this latitude are subject to attack by one brood of maggots which, in normal seasons, injure roots during the latter part of May or early June. As this vegetable is grown in most home gardens these dates closely coincide with the period when radishes are making their most succulent root growth. The results of experiments over a period of four years have shown plainly that a practical means of avoiding losses is to practice early sowing. During some seasons little leeway may be given as to choice of time for planting because of weather and soil conditions; however, it should be the rule to plant seed as soon as the physical condition of the soil permits. For the protection of radishes that are to be harvested during the period of the prevalence of the insects, growing of plants in frames covered with cheesecloth of 20 or 30 mesh has given excellent results. The roots grown by this method have in most seasons given larger yields and have been more succulent and tender than those produced in open beds.

The cherry leaf-beetle (*Galerucella cavicollis* Le Conte) is the subject of Bulletin No. 444. This is a study of the life history, habits and control of a native species which normally feeds on the bird cherry (*Prunus pennsylvanica*). Both insect and plant have the same geographical distribution. The history of the beetle records intermittent attacks on cultivated cherries. The most extensive outbreak of the insect occurred in 1915, and in New York was most serious in the territory west of Cayuga Lake and principally in the Lake Erie Valley, where considerable damage was done in orchards of cultivated cherries.

Seasonal conditions largely govern the life cycle of the insect, especially the development of the pupal stage. Oviposition begins in June and hatching of the eggs occurs during the latter part of July. After hatching the larvæ feed on the foliage, preferably of bird cherry. When compelled to feed on other trees they invariably succumb. Upon completing their growth the larvæ burrow into leaf mold or a short distance into the soil and form cells in which to pupate. The adults begin to emerge during the latter part of August.

Artificial control is affected by arsenicals used in combination with bordeaux mixture and nicotine sulphate, for the use of which directions are given.

The rose leaf-hopper.— Circular No. 55 is a popular treatise, illustrated with two plates and a number of text figures, on the rose leaf-hopper, which is regarded as one of the most destructive insects of roses. The ornamental value of rose plantings is frequently distinctly curtailed, if not actually destroyed, through the activities of this pest. The different life stages are briefly described and figured and seasonal history discussed. It is pointed out that when treated in time the leaf-hopper is not difficult to hold in check. Attention is directed to the selection of safe and efficient spraying mixtures and to the conditions under which plants should be treated in order to obtain satisfactory results. The spray is effective only while the insect is in the immature stages, and the application should be made as soon after hatching as possible.

HORTICULTURAL DIVISION.

Vinifera grapes in New York.— For fifteen years this Station has been experimenting in the culture of Vinifera grapes. Bulletin 432 is a brief account of the experiment. The following is a summary of the work done:

Experimental culture of the European grape was undertaken at this Station in 1902 when cuttings or plants of 19 varieties were received. In 1911 cuttings of more than 70 varieties were received and grafted upon a miscellaneous collection of Station seedlings ranging from 6 to 10 years old. The results were very satisfactory, most of the plants fruiting in 1913.

By giving the vines winter protection and the usual grape sprays they have been kept in a healthy condition.

As a result of the work certain cultural recommendations can be made for New York. One of the most serious difficulties is to secure plants of the desired kinds. Few can be had from eastern nurseries and not a great number from those in California and then not always on resistant roots. For this reason it will frequently be desirable for the eastern grower to know how to graft cuttings on phylloxera-resistant roots such as *Vitis riparia.* This can be done in the nursery row or in the vineyard.

In planting Viniferas less space need be given the vines than with native sorts. Rows six feet apart and plants six feet in the row is a satisfactory distance. Care should be taken that grafted vines do not form roots from the cion.

In the east it is probably best to support the vines with the regular two-wire trellis.

Because of the necessity of bending the trunk to the ground for winter protection, a replacing spur should be left at the base of the trunk to use in forming a new trunk when the old one becomes too stiff. The main trunk should be carried to the lower wire and two fruit canes and two renewal spurs provided for. The young shoots which spring from these canes and spurs grow upright to the second wire when they are pinched off and tied. This gives stockier and more mature canes for the following season.

Cheap winter protection is secured by bending the vines to the ground and covering with a few inches of dirt.

The chief value of the Vinifera grape at present in this State is as a home-garden grape for the amateur, for the commercial grower supplying local markets demanding high quality, and for the plant-breeder seeking to improve the quality of our present varieties.

Most of the Vinifera varieties have originated in regions with a longer season and a much warmer climate than that of New York and many kinds included in the test at Geneva have been discarded because, even in the most favorable seasons, they have not reached maturity.

The varieties are discussed in four groups: (1) Desirable varieties for the grape regions of the State for (a) the table and (b) wine; (2) sorts worthy of testing in the more favorable parts of the State for (a) table and (b) wine; (3) kinds still on probation; (4) varieties of little or no value in the State.

Winter injury of grapes.— Bulletin No. 433 is an account of winter injury of grapes in the winters of 1909–1916 at Fredonia, N. Y., where this Station has been carrying on experiments in vine-growing for the past eight years. The observations and conclusions set forth in the bulletin are as follows:

The data show that approximately half the fruit buds in Concord vineyards about Fredonia were killed during the winter of 1909–10. The injury can be traced to a lack of maturity of the tissues as a result of the sudden termination of the maturing period on October 12, 1909, by unseasonably low temperature. Immaturity is favored by high temperatures and abundant rainfall during late summer and early fall.

The embryo flower clusters may succumb to low temperatures if they enter the dormant period immature and yet the foliage of the bud expand normally. Light crops in years following heavy yields are probably due in part to injury to the floral parts by cold; but unless the extent of the injury be considerable, winter killing is overlooked in explaining the light yield.

The low temperatures of December, 1909, probably killed the buds that were to bear the crop of 1910; but freezing temperatures after April 6, 1910, may also have caused injury or increased it. Following an abnormally warm period in late March and early April came freezing temperatures, and injury may have resulted therefrom to the prematurely stimulated buds.

Winter bud-injury during 1915–16 ranged from 10 to 100 per ct. among the varieties growing on the Station grounds at Fredonia. Injury to Concord varied from 19 to 45 per ct. at the same place. The killing was general thruout the "Grape Belt." This great loss may have been due to unfavorable climatic conditions during the maturing months of August, September and October, 1915, as indicated by the poor ripening of the fruit of 1915, which was shown by the low sugar and high acid content. During the week of January 22–29, 1916, however, the average hourly temperature for 96 consecutive hours was 52.6 degrees, which no doubt awakened growth activity at this time; while the minimum temperature of the winter, —16 degrees, occurred in March. The winter killing of 1915–16 may have taken place, therefore, either shortly after the high temperatures of January, or not until the low ones of March.

The fertilizer elements, nitrogen, phosphorus and potassium, did not affect maturity and hence did not, apparently, influence the degree of killing. Extensive injury is closely correlated with poorly drained soils, altho bud killing occurred where the drainage conditions were satisfactory. Severe pruning after late frost injury of spring has apparently indirectly favored bud killing thru inducing rank wood growth.

Resistance to low temperatures is probably a species character and is possibly correlated with the hardness of wood.

Culture of the globe artichoke.— For several years the globe artichoke has been successfully cultivated on the Station grounds.

Bulletin 435 gives an account of the methods practiced with some general observations regarding the plant. The chief facts set forth are as follows:

The plant is not fully hardy in our latitude and requires covering to endure winter. Coal ashes proved to be a satisfactory material with which to cover the artichoke, affording sufficient protection without causing decay of the crowns.

From records taken, it is evident that marked variations exist in the producing capacity of individual plants of a variety. Consequently, the separation of the offshoots from the old main root of the plant is the most reliable method of propagation and should materially assist in the establishment of uniform and meritorious strains.

The edible portion of the artichoke is the flower bud.

The plant itself is remarkably thrifty in growth and is practically free from fungus and insect pests. One insect, a black aphid or louse, becomes troublesome at times but is satisfactorily controlled by spraying with a properly prepared dilution of Black Leaf 40 and whale-oil soap.

Orchards: Location and care.— Circular 52 gives instructions for the care of orchards of tree fruits. The circular is a brief treatise on the culture of all tree fruits. The following are the chief topics discussed: Location, orchard plans, planting, cultivation, cover-crops, inter-crops, fertilizers, pruning, pests, spraying outfits, spraying formulas, spraying schedules, grafting and thinning.

INSPECTION WORK.

The Station examines samples of several classes of material subject to inspection by the Commissioner of Agriculture, among which are agricultural seeds, insecticides and fungicides, commercial fertilizers and concentrated feeding stuffs. All glassware used where milk and cream are paid for by the Babcock test must also be examined and marked at the Station.

This inspection work is extensive and time-consuming as it receives a large share of the attention of six chemists, an assistant botanist and his laboratory assistant, and one man in the Dairy Division.

The figures given below give an idea of the scope of these lines of Station activity during 1917:

BABCOCK GLASSWARE TESTED.

Ten per ct. bottles	12,585	17.6 pipettes	3,069
Eight per ct. bottles	10,928	9 cc. pipettes	143
Thirty per ct. 9-gram	72	8.8 cc. pipettes	12
Thirty per ct. 18-gram	581	6 cc. pipettes	12
Forty per ct. 18-gram	301	18 cc. pipettes	83
Fifty per ct. 18-gram	1,104	Skimmilk bottles	179
Fifty per ct. 9-gram	2,569	Acid measures	562
Fifty per ct. 9-inch 9-gram	182		
Sixty per ct. 6-inch 9-gram	432	Total	33,192
Thirty per ct. 9-inch 9-gram	378		

SAMPLES OF SEED EXAMINED.

Official samples	340
Samples sent by farmers and other correspondents	476

FERTILIZER SAMPLES ANALYZED.

During the year 705 samples of fertilizers were analyzed. There were 265 samples of complete fertilizers; 249 samples of mixed fertilizers, containing nitrogen and phosphoric acid; 69 samples of acid phosphate; 29 samples of calcium or lime compounds; 37 samples of bone; 16 samples of tankage; 13 samples of nitrate of soda; 8 samples of dried animal manures; and a small number of samples each of blood, dissolved bone, mixtures of phosphoric acid and potash, insoluble phosphoric acid materials, ashes, garbage tankage, ground fish, soot, mixtures of nitrogen and insoluble phosphoric acid; and mixtures of calcium compounds and phosphoric acid.

FEEDING-STUFFS SAMPLES ANALYZED.

During the year 1917, 703 samples of feeding-stuffs were analyzed. There were 38 samples of cottonseed meal, 13 of linseed meal, 8 of malt sprouts, 18 of distillers' dried grains, 2 of yeast or vinegar dried grains, 10 of brewers' dried grains, 14 of corn gluten feed and meal, 22 of hominy feed, 107 of compounded feeds, 113 of molasses compounded feeds, 92 of compounded poultry foods, 1 of calf meals, 51 of animal products, 26 of alfalfa meal, 44 of wheat bran, 52 of wheat middlings, 21 of wheat bran and wheat middlings, 1 of wheat bran and low-grade wheat flour, 3 of ground corn and oats, 5 of wheat bran and corn by-products, screenings, 19 of corn meal and of corn feed meal, 7 of rye by-products, 2 of ground screenings, and 10 of miscellaneous mixtures.

PUBLICATIONS ISSUED DURING 1917.

Bulletins.

No. 429. February. Goat's milk for infant feeding. W. H. Jordan and G. A. Smith. Pages 20, plates 2.
Popular edition, page 7.

No. 430. March. Ground limestone for use in New York State. J. F. Barker and W. W. Baer. Pages 12, plates 4, map 1.

No. 431. March. Plant lice injurious to apple orchards, II; Studies on control of newly hatched aphides, II. P. J. Parrott, H. E. Hodgkiss and F. H. Lathrop. Pages 47, color plate, 1, figs. 4.
No popular edition issued.

No. 432. April. Vinifera grapes in New York. R. D. Anthony under direction of U. P. Hedrick. Pages 25, color plate 1, plates 4.
Popular edition, pages 7.

No. 433. April. Winter injury of grapes. F. E. Gladwin. Pages 33, plates 8.
Popular edition, pages 7, plates 2.

No. 434. May. Inspection of feeding stuffs. Pages 169.
No popular edition issued.

No. 435. May. Culture of the globe artichoke. J. W. Wellington, under direction of U. P. Hedrick. Pages 4, plates 2, figs. 3.
No popular edition issued.

No. 436. June. Blackheart and the aeration of potatoes in storage. F. C. Stewart and A. J. Mix. Pages 42, plates 10.
Popular edition, pages 11, plates 4.

No. 437. July. Neck-rot disease of onions. M. T. Munn. Pages 93, plates 11.
Popular edition, pages 8.

No. 438. November. What is meant by " quality " in milk? R. S. Breed, H. A. Harding, W. A. Stocking, Jr., and E. G. Hastings. Pages 19.
No popular edition issued.

No. 439. November. The number of bacteria in milk. James D. Brew and W. D. Dotterrer. Pages 45.
Popular edition, pages 12.

No. 440. December. Analyses of samples of commercial fertilizers collected by the Commissioner of Agriculture during 1917. Pages 62.
No popular edition issued.

No. 441. December. Inspection of feeding stuffs. Pages 107. No popular edition issued.

No. 442. December. The radish maggot. P. J. Parrott and H. Glasgow. Pages 23, plates 8, figs. 2.
Popular edition, pages 4, plates 2.

No. 443. December. The control of the bacterial quality of market milk by direct microscopic examination. Robert S. Breed and James D. Brew. Pages 30.
Popular edition, pages 12.

No. 444. December. The cherry leaf-beetle. F. Z. Hartzell. Pages 76, plates 8, figs. 8.
No popular edition issued.

No. 445. December. Director's report for 1917. W. H. Jordan. Pages 24.
No popular edition issued.

Technical Bulletins.

No. 57. January. Soil flora studies: I. The general characteristics of the microscopic flora of soil. II. Methods best adapted to the study of the soil flora. H. Joel Conn. Pages 42.

No. 58. March. Soil flora studies: III. Spore-forming bacteria in soil. H. Joel Conn. Pages 16, figs. 4.

No. 59. March. Soil flora studies: IV. Non-spore-forming bacteria in soil. H. Joel Conn. Pages 18.
No. 60. March. Soil flora studies: V. Actinomycetes in soil. H. Joel Conn. Pages 25.
No. 61. March. Facilities for lysimeter and out-door pot culture work at the station. J. F. Barker. Pages 10, plates 2, figs. 2.
No. 62. May. Determination of carbonates in limestone and other materials. J. F. Barker. Pages 7, fig. 1.
No. 63. September. Inheritance of sex in strawberries. R. D. Anthony under direction of U. P. Hedrick. Pages 10.

CIRCULARS.

No. 52. May 20. Orchards: Location and care. U. P. Hedrick. Pages 16, plates 2.
No. 53. May 1. Culture of field beans. J. W. Wellington. Pages 4.
No. 54. May 10. Milking machines. Pages 4.
No. 55. May 10. The rose leaf-hopper. F. H. Lathrop. Pages 4, plates 2, figs. 3.

W. H. JORDAN.

NEW YORK AGRICULTURAL EXPERIMENT STATION,
GENEVA, N. Y., January 15, 1918.

REPORT

OF THE

Department of Agronomy.

[1] J. F. Barker, *Agronomist.*
[2] Reginald C. Collison, *Agronomist.*
[3] James E. Mensching, *Associate Chemist (Agronomy).*
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II. Facilities for lysimeter and out-door pot work at the Station.
III. Determination of carbonates in limestone and other materials.

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[3] Appointed September 1, 1917.
[4] Absent on leave after July 1, 1917; in United States Naval Service.

REPORT OF THE DEPARTMENT OF AGRONOMY.

GROUND LIMESTONE FOR USE IN NEW YORK STATE.*

J. F. BARKER AND W. W. BAER.

Five years ago we could learn of but one company in this State producing ground limestone for the agricultural trade and there was one producing marl. On the basis of the merits of this material its demand has increased rapidly until now there are 56 plants in the State engaged in the production and more than a dozen plants outside make extensive shipments to New York farmers. The 56 plants mentioned have a possible capacity of approximately 675,000 tons annually, counting only eight months of the year and a working day of eight to ten hours.

PURPOSE OF THIS PUBLICATION.

When the present fertilizer inspection law was written, about the only lime of agricultural interest on the market was high grade calcium burned lime used more for spraying purposes than for application to the soil. Accordingly provision was made for inspection of such lime material only as sold for $5 or more per ton and for the determination of calcium alone. With the large volume of ground limestone now on the market, the greater portion of which sells away below the above figure, there has arisen a demand for information regarding the relative value of different products, not furnished by the fertilizer inspection work. Bulletin No. 400 (March, 1915) and the present bulletin have been intended to fill this need. In the preparation of this report aid has been rendered by the County Farm Bureau men, who have collected samples. The data given herein represent the analyses of samples taken by these men and by representatives of this Station during the season of 1916. In a few cases, indicated in the tables, use has been made of data from Bulletin No. 400 where the authors have reason to believe it represents the character of the present product.

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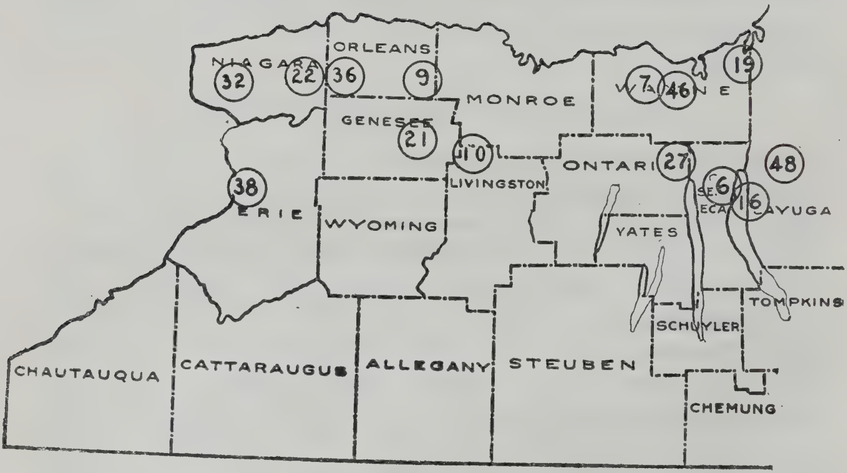
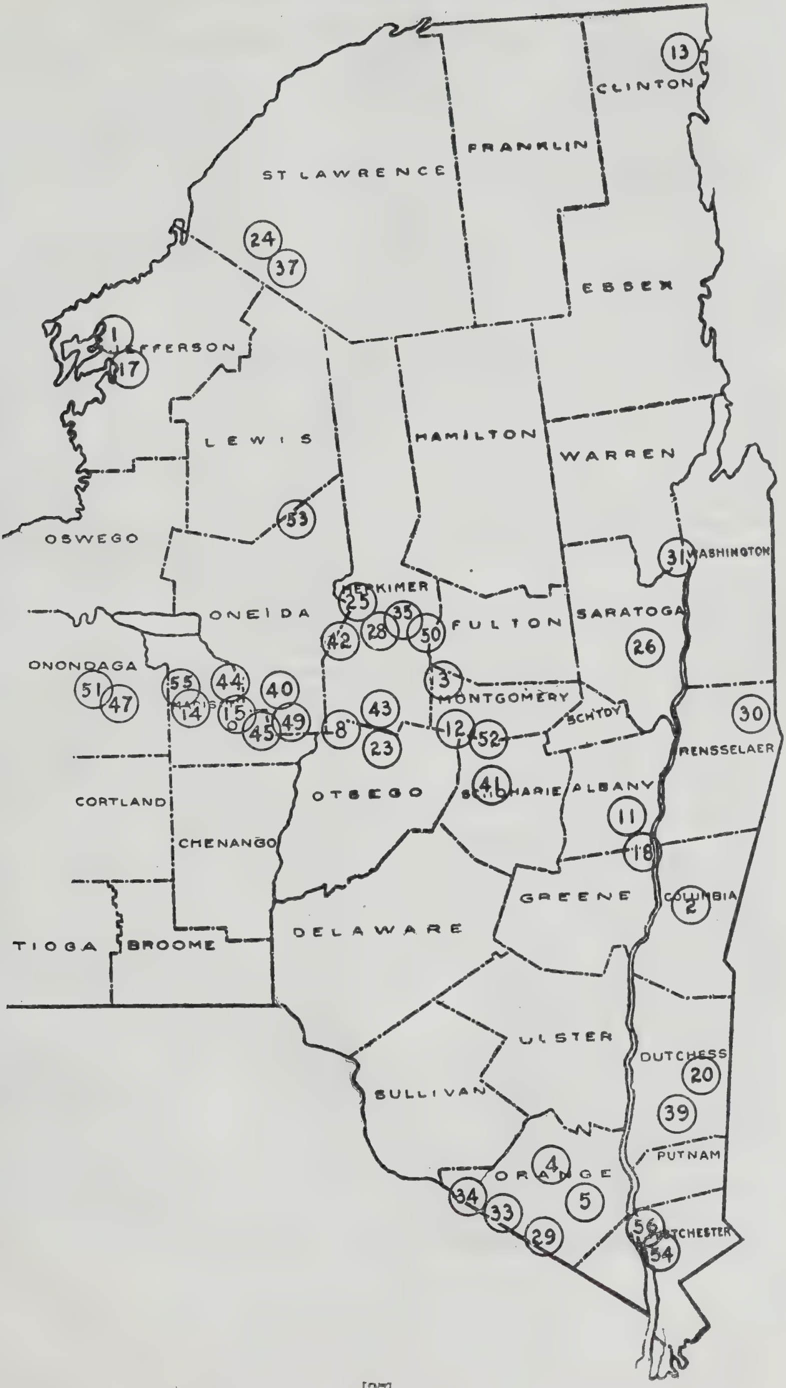
* A reprint of Bulletin No. 430, March, 1917.

Map Showing Distribution of Plants in New York State Producing Ground Limestone.

(Numbers correspond with those in Table I.)

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CORTLAND
CHENANGO
OTSEGO
SCHOHARIE
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TIOGA
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TABLE I.—LIMESTONE GRINDIG PLANTS IN NEW YORK STATE, WITH ANALYSIS OF PRODUCT.

NAME OF COMPANY	Post office address	Map No.	Location of quarry	Calcium carbonate, $CaCO_2$	Magnesium carbonate, $MgCO_3$	Total carbonates	Calcium carbonate equivalent
Adams & Duford Co....	Chaumont......	1	Chaumont......	90.2	2.2	92.4	**92.6**
*B. & B. Lime & Stone Co.................	Mellenville.....	2	Mellenville.....	70.3	7.7	78.0	**79.4**
Burkdorf, H. J.........	St. Johnsville...	3	St. Johnsville...	92.1	1.0	93.1	**93.3**
Booth Brothers.........	Goshen.........	4	Campbell Hall..	52.3	32.2	84.5	**90.7**
Bull, James............	Monroe........	5	Monroe........	58.1	37.4	95.5	**102.6**
Bishop, Tunis..........	Seneca Falls....	6	Fayette........	71.7	15.1	86.8	**89.7**
Butts Bros............	Sodus..........	7	Sodus..........	62.5	33.1	95.6	**101.8**
Brewer, Henry.........	W. Winfield....	8	Cedarville......	85.0	1.0	86.0	**86.2**
Cole, R. J.............	Clarendon......	9	Clarendon......	51.3	41.2	92.5	**100.4**
Caledonia Chemical Co..	Caledonia......	10	Caledonia......	84.1	1.3	85.4	**85.6**
Callanan Road Imp. Co.	So. Bethlehem..	11	So. Bethlehem...	79.1	4.1	83.2	**84.0**
Canajoharie Stone Co...	Canajoharie....	12	Canajoharie....	78.8	6.6	85.4	**86.7**
Chazy Marble & Lime Co.	Chazy..........	13	Chazy.........	91.1	2.2	93.3	**93.7**
Conley Stone Co.......	Utica..........	14	Blakeslee.......	94.2	1.5	95.7	**96.0**
Conley Stone Co.......	Utica..........	15	Munnsville.....	92.4	2.0	94.4	**94.8**
Cayuga Limestone Co...	Union Springs...	16	Union Springs...	69.6	3.6	73.2	**73.9**
Case, Holley...........	Dexter.........	17	Limerick.......	99.7	Trace	99.7	**99.7**
Day, Stephens.........	Coeymans Hollow	18	Coeymans Hollow	93.2	1.7	94.9	**95.3**
Doud & Teachout......	Red Creek......	19	Red creek......	72.2	21.9	94.1	**98.3**
*Dutchess Co. Lime Co.	Dover Plains....	20	Dover Plains....	53.8	34.0	87.8	**94.1**
LeRoy Lime Co........ Eastern Lime & Fert. Co.	LeRoy....... Franklinville..	21	LeRoy.........	89.4	1.1	90.5	**90.7**
Eastern Lime & Fert. Co.	Franklinville....	22	Gasport........	55.7	35.4	91.1	**97.9**
*Fretz, M. W..........	Richfield Springs	23	Richfield Springs	89.7	.4	90.1	**90.2**
Gouverneur Limestone Co..................	Gouverneur.....	24	Gouverneur.....	90.0	4.5	94.5	**95.3**
Grant, Carry...........	Poland.........	25	Poland.........	87.6	Trace	87.6	**87.6**
Gailor Stone Co........	Saratoga Springs	26	Saratoga Springs	58.7	29.33	88.0	**93.6**
Geneva Limestone Co...	Geneva........	27	Oaks Corners...	57.5	27.8	85.3	**90.6**
Harris, John...........	Middleville.....	28	Middleville.....	96.6	Trace	96.6	**96.6**
Horton, M. V. B.......	Warwick.......	29	Warwick.......	51.8	42.4	94.2	**102.2**
Hoosick Lime Co.......	Hoosick........	30	Hoosick........	97.9	.9	98.9	**99.0**
Jointa Lime Co.........	Glens Falls.....	31	Glens Falls.....	95.5	.7	96.2	**96.3**
Langdon & Co.........	Elmira.........	32	Pekin..........	79.8	4.6	84.4	**85.4**
Lain, M. A............	Westtown......	33	Westtown......	53.1	32.5	85.6	**91.8**
Leher, M. M...........	Port Jervis.....	34	Port Jervis.....	80.1	2.7	82.8	**83.3**
Mahardy & Murphy....	Middleville.....	35	Middleville.....	92.6	2.0	94.6	**95.0**
*Medina Limestone Co..	Medina........	36	Medina........	53.7	43.3	97.0	**105.1**
Maxner Quarry.........	Gouverneur.....	37	Gouverneur.....	89.0	6.4	95.4	**96.6**
Michigan Limestone & Chemical Co.........	Buffalo.........	38	Grinding plant in Buffalo.......	97.3	.8	98.1	**98.2**
McKinney, Glen Ford...	52 William st., N. Y. city....	39	Clove Valley....	60.2	31.5	91.7	**97.7**

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* Analyses based mainly on data given in Bulletin No. 400.

TABLE I (*concluded*).

NAME OF COMPANY	Post office address	Map No.	Location of quarry	Calcium carbonate, $CaCO_2$	Magnesium carbonate, $MgCO_3$	Total carbonates.	Calcium carbonate equivalent.
McAdam, Quentin	Utica	40	Deansboro	No sample obtained.			
Norton Stone & Lime Co.	Cobleskill	41	Cobleskill	80.3	1.6	81.9	**82.3**
†Newport Stone Co.	Newport	42	Newport	88.2	1.6	89.8	**90.1**
Southern N. Y. Power & Railway Corporation	Cooperstown	43	Cullen	84.8	1.0	85.8	**86.0**
Peck, A. S.	Valley Mills	44	Valley Mills	No sample obtained.			
Putnam Quarries	Oriskany Falls	45	Oriskany Falls	94.8	....	94.8	**94.8**
Plumber, W. H.	Sodus	46	Sodus	52.8	43.3	96.1	**104.5**
Rock Cut Stone Co.	Syracuse	47	Rock Cut	71.5	13.6	85.1	**87.6**
Rock Cut Stone Co.	Syracuse	48	Auburn	77.6	1.8	79.4	**79.8**
Salisbury, W. A.	Waterville	49	Waterville	91.3	....	91.3	**91.3**
*Sasman, Christopher	Salisbury Center	50	Salisbury Center	89.7	....	89.8	**89.8**
Solvay Process Co.	Syracuse	51	Jamesville	87.6	1.3	89.9	**89.2**
*Smith, N. T.	Sharon Springs	52	Sharon Springs	91.4	.9	92.3	**92.5**
*Sugar River Stone Co.	Boonville	53	Boonville	90.9	1.3	92.2	**92.5**
*Upper Hudson Stone Co.	26 Courtland st., New York	54	Verplanck Point.	55.1	33.2	88.3	**94.6**
Worlock Stone Co.	Canastota	55	Blakeslee	86.9	4.1	91.0	**91.8**
Westchester & Putnam Lime Company	Peekskill	56	Peekskill	50.5	41.3	91.8	**99.7**

* Analyses based mainly on data given in Bulletin No. 400.
† Average run of quarry stone.

TABLE II. — SOME COMPANIES IN NEAR-BY STATES SHIPPING LIMESTONE INTO NEW YORK STATE.

NAME OF COMPANY	Postoffice address	Calcium carbonate $CaCO_2$	Magnesium carbonate $MgCO_2$	Total carbonates	Calcium carbonate equivalent
Bessemer Limestone Co.	Youngstown, Ohio	85.3	1.6	86.9	**87.2**
*Baker, J. E., Company	Bainbridge, Pa.	86.7	5.8	92.5	**93.6**
*Carbon Limestone Co.	Youngstown, Ohio	88.9	1.2	90.1	**90.2**
Clydesdale Lime & Stone Co.	Pittsburg, Pa.	No sample obtained.			
Edison Portland Cement Co.	Stewartsville, N. J.	86.4	2.5	88.9	**89.4**
Grangers Lime & Marble Co.	West Stockbridge, Mass.	89.7	2.8	92.5	**93.0**
Kelley Island Lime Co.	Cleveland, Ohio	79.4	16.0	95.4	**98.5**
McKeefrey & Co.	Leetonia, Ohio	86.1	1.7	87.8	**88.2**
New England Limestone Co.	Adams, Mass.	97.4	.3	97.7	**97.7**
Norwich Chemical Co.	Buffalo, New York	No sample obtained.			
Pownal Limestone Co.	North Pownal, Vermont.	No sample obtained.			
Stearns Lime Co.	Danbury Co.	69.6	16.0	85.6	**88.6**
Vermont Marble Co.	Rutland, Vermont	No sample obtained.			

COMPARATIVE VALUE OF GROUND LIMESTONES.

Limestones are made up of calcium carbonate or calcium and magnesium carbonates and a varying amount of clay-like material. The better grades run about 90 per ct. total carbonates. All limestones contain a higher percentage of calcium carbonate ($CaCO_3$) than magnesium carbonate ($MgCO_3$), the latter varying in different stones from nothing to a maximum of about 45 per ct. Those containing ten per ct. or more magnesium carbonate are usually referred to as *magnesium limestone* and if they carry near the maximum amount they are also called *dolomites*.

So far as any study has been made of the subject, magnesium limestones have been found to be fully as effective as straight calcium stones for practical use in soil improvement. In fact, a given weight of magnesium carbonate is capable of neutralizing somewhat more acidity than the same weight of calcium carbonate, 100 pounds of the former being equal to 119 pounds of the latter in this respect. Also, it is to be remembered that magnesium as well as calcium is one of the ten essential elements of plant food and that a part of the benefit from an application of limestone often may be due to the more liberal supply of these two elements of fertility.

The comparative value of different limestones for use on the soil is conveniently expressed in terms of their *calcium carbonate equivalents*. This figure is obtained by adding to the percentage of calcium carbonate the percentage of magnesium carbonate multiplied by 1.19, since one pound of magnesium carbonate is chemically equivalent to 1.19 pounds of calcium carbonate. Thus, a stone containing 76 per ct. calcium carbonate and 20 per ct. magnesium carbonate (equal to 23.8 per ct. calcium carbonate) would have a calcium carbonate equivalent of 99.8. This figure will usually be but little different from that for the percentage of total carbonate.

The buyer of ground limestone is often confused by long and varied statements of analyses and by claims to special merit made for particular products. He should note the figure for calcium carbonate equivalent and bear in mind that, assuming reasonable fineness, this gives the comparative value of the product. If a stone showing a calcium carbonate equivalent of 90 can be delivered to the buyer's railway station for $1.80 per ton, then one having an equivalent of 100 is worth scarcely more than $2.00 per ton; the two are valuable in the proportion of 90 to 100, allowing only

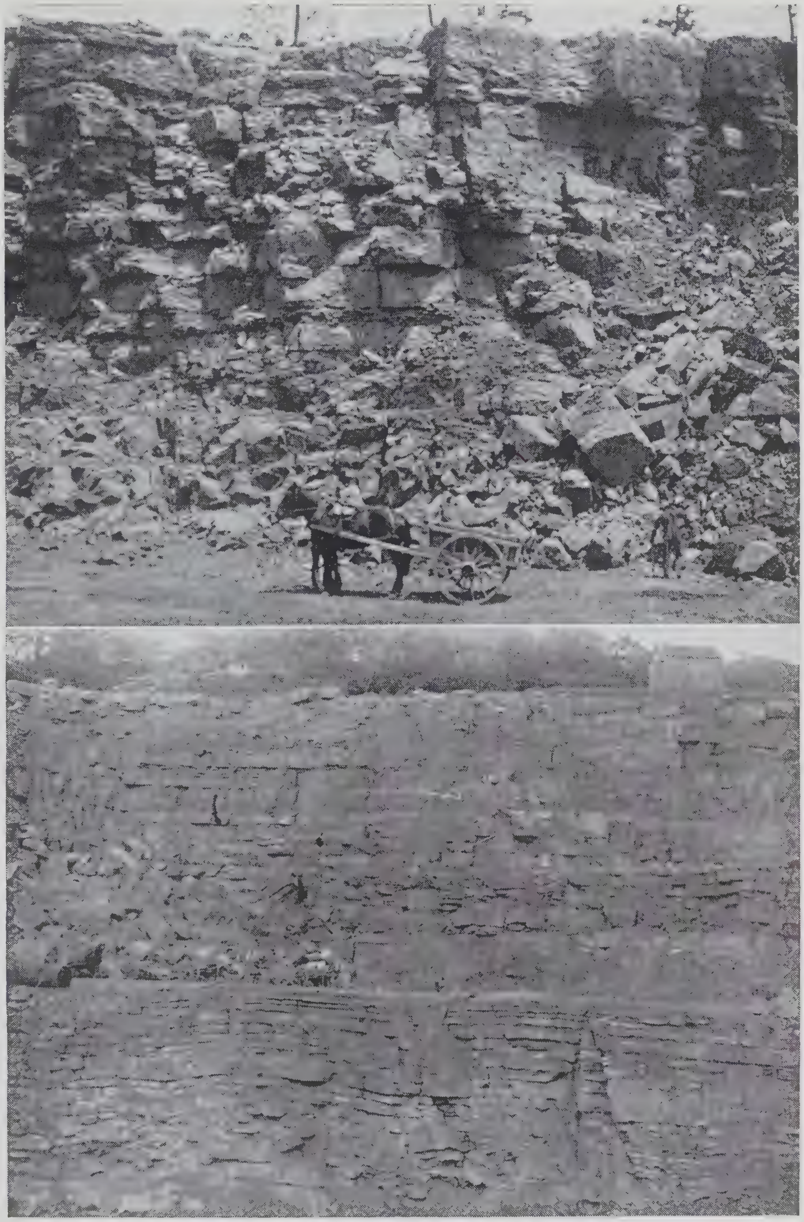


Plate I.—Enormous Deposits of Excellent Limestone are Available for Use in New York State.

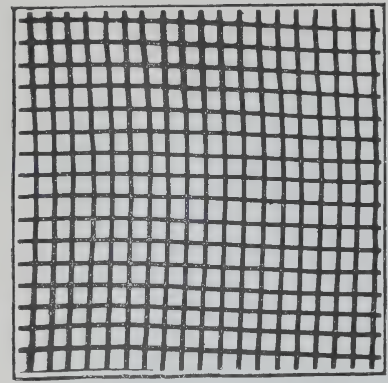
Plate II.
1.—Modern Limestone Grinding Plant.
2.—Drawing Limestone to the Grinder.

Plate III.

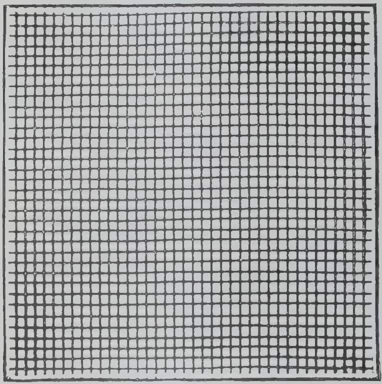
Community Limestone Grinding Plant.

(Courtesy Orange County Farm Bureau.)

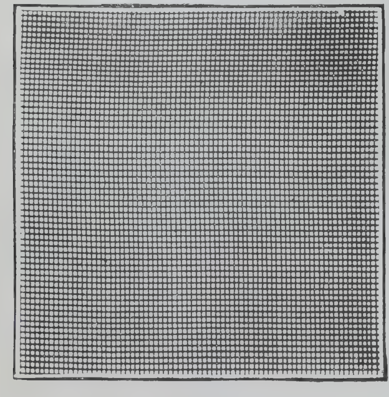
Soluble Effect of Underground Water on Limestone.



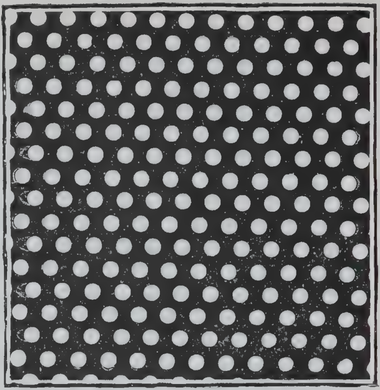
1 10-Mesh sieve.



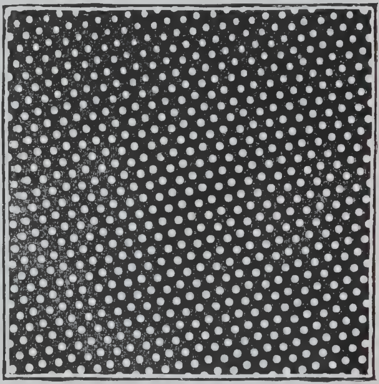
2 20-Mesh sieve.



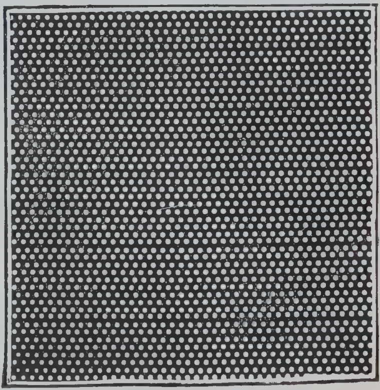
3 40-Mesh sieve.



4 2-Millimeter sieve.



5 1-Millimeter sieve.



6 ½-Millimeter sieve.

Plate IV.—Sieves Used in Testing the Degree of Fineness of Limestone.

for the slightly greater amount of work in drawing and applying a given quantity of lime in the lower grade stone.

For a classification and description of the limestone formations of the State together with a map showing extent and location of same the reader is referred to Technical Bulletin No. 47 of this Station.

The usual method of limestone analysis determines total calcium and magnesium without regard to the form in which they exist in the stone, it being taken for granted that all is in the carbonate form. The methods used in obtaining the analyses in this bulletin take account only of the carbonates in limestone, the part effective for neutralizing acidity, and we have found that the figures for calcium carbonate equivalent obtained in this way may run on different stones from 0 to 3 per ct. lower than would be shown by total analyses. But usually there is little or no difference.

FINENESS.

In referring to degrees of fineness of ground limestone reference is often made to the percentages passing different grades of sieves such as are shown in exact sizes on Plate IV. Sieves called 10-mesh, 20-mesh, 40-mesh, etc., have as many openings to the linear inch; 2, 1 and ½ millimeter sieves have round holes of such diameters, as 2, 1, or ½ millimeter, 25.3 millimeters being equal to one inch.

The subject of fineness is more fully discussed in Bulletin No. 400 of this Station; here let us say only briefly:

Farmers who have had experience with the use of ground limestone are as a rule satisfied with only a reasonable degree of fineness, and are able to judge the material by inspection. When limestone is ground so the entire product will pass a 10-mesh, or 2 mm., sieve, the greater part of it will be finer than a 40-mesh, or ½ mm., sieve, It is then as fine as the ordinary bone meal on the market sold as " fine ground bone." It is an established fact that the finer bone is ground the more readily " available " as a fertilizer, yet in the practical working out of the problem the above degree of fineness is accepted as satisfactory. In this condition it is a favorite form of phosphorus fertilizer, the only objection being the very limited quantity and consequently high price. Fineness in bone meal is more important than in limestone for it is used at 200 to 1000 pounds per acre and costs $20 to $40 a ton, while limestone is used at the rates of 2000 to 10,000 pounds per acre and the farmer pays $1.50

to $4.00 per ton. Moreover, calcium phosphate, bone meal, is less soluble under soil conditions than calcium and magnesium carbonates, limestone.

In the last few years limestone has almost entirely replaced all forms of burned lime for use in soil improvement and has done so on the basis of material of only medium fineness, such as described above.

There are now in operation in this State more than a dozen small portable community grinders; they are doing much to help solve the ground limestone problem and their use is rapidly increasing. In the practical operation of these machines they grind only to medium fineness (2 mm.). To insist upon extreme fineness is to discourage their use.

For limestone ground as fine as cement the farmer must pay fifty cents to $1.50 more per ton than for the medium ground, also it must be handled in sacks, which adds at least another dollar per ton. There is no good evidence that in practical use the proportionately smaller amount of such material his money buys will satisfy the farmer's lime-hungry soil as well as the larger ration of reasonably fine limestone. To meet the demands of eastern soils for lime at the present time we need an abundant and easy supply of that material and its production and use must not be delayed by academic considerations.

SOILS IN NEW YORK STATE IN NEED OF LIMING.

Three-fourths or more of the farm lands in New York State would be greatly benefited by a liberal application of lime in some form. They vary in the amount of limestone required to satisfy their acid conditions from 1 to 10 tons or more per acre. Just what are the corresponding amounts of limestone most profitable to use is another question. From such experimental evidence as is at hand it seems probable we should use two to four tons per acre as an initial application, the larger amount on the more acid soils or for crops responding most favorably to the use of lime.

The use of limestone on acid soils is fundamental to any systematic method for their improvement. And this is becoming to be so generally recognized and is a matter of such frequent discussion that it need not be advocated at length here.

The soils in this State not at present in need of liming are those which have been naturally well stocked with limestone by reason of their having been derived in large part from limestone formations, although in regions where these occur there are now frequent areas in need of lime. However, in any community where alfalfa is commonly grown without difficulty it can safely be assumed that additional limestone is not much needed. Technical Bulletin No. 47 of this Station gives some indications as to where natural limestone soils are likely to be found. Sections of the State especially in need of liming compose nearly all of the southern half (excepting parts of Schoharie, Albany, Dutchess, Putnam, Westchester and Orange counties), the Lake Front lands bordering Lake Erie and Lake Ontario, nearly all of Long Island and a large portion of the rest of the State. These areas represent at least 10,000,000 acres of improved farm land and at lowest calculation should have one ton of limestone every four years, 2,500,000 tons annually. Since only about one-tenth of this amount has yet been used in this State in one year, it is plain that the practice of liming the land is now in its early stages.

FIELD TESTS FOR THE NEED OF LIME.

Speaking for New York State: Soils derived from siliceous rocks are in need of liming. Such rocks include sandstones, sandy shales, clay shales, granites, trap, gneiss, schists and others. Soils derived mainly from limestone are little or not at all in need of liming. Where the growing of red clover has become difficult the soil is probably in need of liming. The prevalence of sorrel or paintbrush usually indicates a distinctly acid soil. Soils with a light color, gray, grayish brown, or yellowish shade, are usually in need of liming. Soils which turn blue litmus paper pink are nearly always in need of liming.

The litmus-paper test for soil acidity may be made as follows: Form a ball of wet soil about the size of the fist, break it open and insert a double thickness of blue litmus-paper (obtainable at drug store). Press the ball firmly together and allow to stand as much as a half hour. If at the end of this time the sides of the paper in contact with each other have changed to a distinctly pink color there is positive evidence of acidity and it may safely be assumed that benefit will follow liming.

It is now nearly always possible to tell in advance whether or not a soil is in need of liming without waiting for the slow and expensive method of trying out an application of limestone in the field. Besides, one year's trial may often fail to give conclusive results.

COMPARATIVE RESPONSE OF CROPS TO LIMING.

Plants vary greatly in regard to their sensitiveness to an acid condition of the soil, but with only a few exceptions all agricultural crops are distinctly benefited by the use of limestone when grown on such lands. There are a rather large number of crops that can scarcely be grown with success on lands only moderately acid. Without doubt the best evidence on record showing the comparative effect of liming on a large number of crops grown on the same land is that published by the Rhode Island Station in Bulletin No. 160. The following table sums up this data for those crops of greater agricultural interest. The soil was distinctly in need of lime and both the limed and unlimed land was liberally fertilized with nitrogen, phosphorus and potassium. The figures given represent the yield with fertilizer and limestone as compared with a yield of 100 for fertilizer alone. Thus 104 shows a 4 per ct. increase and 200 shows a 100 per ct. increase. Where 300 + is given it indicates that there was at least 200 per ct. increase but in such cases the difference may often be due to a practical failure on the unlimed land and so the exact figures for comparisons might be misleading.

TABLE III.— COMPARATIVE RESPONSE OF DIFFERENT CROPS TO LIMING ON A BASIS OF 100 FOR THE YIELD OF UNLIMED LAND.

Summarized from data in Bulletin No. 160 of Rhode Island Experiment Station.

Cranberry	20	Lima beans	120	Sweet clover	170
Sorrel	62	Pop corn	120	Alfalfa	172
Watermelon	72	Turnip	122	Cabbage	180
Serredella	75	Rhubarb	123	Rape	200
Peanut	80	Raspberry	125	Horse radish	200
Cow pea	83	Soy beans	127	Broccoli	200
Field corn	100	Sunflower	128	Muskmelon	225
Millet	100	Carrots	130	Cauliflower	230
Vetch	100	Brussels sprouts	130	Chard	230
Rye	100	Tomato	133	Currants	240
Radish	102	Cucumber	140	Onions	300+
Fruit trees	103	Barley	146	Parsnips	300+
Alsike clover	104	Sweet corn	150	Spinach	300+
Gooseberry	104	Kale	150	Asparagus	300+
Grapes	105	Beans, red	150	Beets	300+
Potato	106	Red clover	156	Mangels	300+
Wax bean	106	Hay, mixed	156	Lettuce	300+
Strawberries	110	Koh rabi	158	Celery	300+
Squash	112	Peas	160	Leek	300+
Blackberry	115	Sorgum	160	Salsify	300+
Oats	120	Buckwheat	160		
Timothy	120	Pumpkin	163		

The test on a crop was each year made in duplicate, limed and unlimed with nitrogen applied as sulphate of ammonia and limed and unlimed with nitrogen as nitrate of soda. The yield on the unlimed land with sulphate of ammonia was usually much lower than with nitrate of soda, but in preparing this summary the results in connection with nitrate of soda only were used, since sulphate of ammonia has been a rather uncommon fertilizer in New York State. The figures nearly all represent the average of two or more seasons' tests, usually four or more, and often include more than one variety of the crop. Since these tests were made only upon soil having a rather limited range of physical and chemical characteristics it is not assumed that the relative effect of liming for these crops will within narrow limits always be the same as in this case; but the figures do represent the best comparative data on record at present.

NATURE OF SOIL ACIDITY.

In natural soil processes acids are constantly being formed incidental to the decay of organic matter. These acids consist of carbonic acid, various organic acids and the stronger nitric acid (HNO_3). All have ability to combine with and remove from action the bases present in the soil. The formation of organic acids is familiar to everyone in the souring of milk, in the production of sauerkraut, in the fermenting of silage, and in the manufacture of wine and vinegar from fruit juices. Nitric acid is formed from the nitrogen of organic matter by the process of nitrification. In the production of almost any ordinary crop as much as 100 pounds of nitrogen per acre is converted into nitric acid. Under favorable conditions 200 pounds or more of nitrogen per acre are commonly transformed into nitric acid. One hundred pounds of nitrogen forms 450 pounds of nitric acid which is capable of using up 357 pounds of calcium carbonate. The carbonic acid and organic acids produced at the same time would be capable of using up a much larger quantity of limestone than this. If carbonates are not present to combine with these acids they attack the silicate compounds of the soil and extract from them their basic constituents, such as calcium, magnesium, sodium and potassium. Acids are added to the soil to some extent by the use of many commercial fertilizers. When 500 pounds of acid phosphate are applied to the soil enough acid is introduced to use up about 200 pounds of limestone. By the use of ammonium sulphate $(NH_4)_2SO_4)$ a small amount of sulphuric acid (H_2SO_4) is formed, as the plants use up the ammonia and leave the free sulphuric acid behind. Field experiments show that plats treated with ammonium sulphate become acid sooner than plats alongside treated with a similar amount of nitrogen in nitrate of soda or dried blood. When free sulphur is added to the soil, as in the dusting of plants for fungus diseases, it is soon oxidized to sulphuric acid and relatively large quantities of acid may be formed in this way.

It should be said in regard to organic acids that they may be largely oxidized, burned up, in the soil before they have exerted their effect. Also, if after combining with a base an insoluble compound is formed, as is often the case especially with compounds of calcium, this will gradually undergo oxidation leaving a basic ash to neutralize other acidity. In this way unleached manure has a tendency to reduce soil acidity; also the plowing under of green manures may finally help overcome acidity in surface soils as compared with land from which crops have all been removed.

It is not correct to say that acid soils are due to an accumulation of acids, such as described above, for hardly an appreciable amount of such acids are ever present in the free state in any soil. But the acids remove the bases from the soil, first the carbonates and then the basic constituents of the silicates; and when this process has gone on long enough the soil becomes deficient in basic materials. It is then potentially acid; that is, it behaves as an acid in contact with basic materials or a compound like limestone which readily parts with it basic constituents in the presence of even very weak acids.

FACILITIES FOR LYSIMETER AND OUT-DOOR POT CULTURE WORK AT THE STATION.*

J. F. BARKER.

DESCRIPTION OF LYSIMETERS.

A battery of twenty lysimeters was installed at this Station during the summer and fall of 1914. The diagrams and plates herein contained show the construction of this equipment without need of much detailed description.

The tanks are of 3/16-inch steel with riveted seams. They are four feet eight and one-half inches inside diameter, and 16 of them are four feet deep, 2 have a depth of eight feet and 2 a depth of two feet. Each has a conical bottom allowing in the center 6 inches additional depth. The surface area of soil exposed in each tank gives .0004 of an acre. Heavy coatings of pitch protect the tanks inside and out from rust and they are securely set in puddles of concrete. A two-inch bronze pipe passes from the bottom of each tank to the underground compartment or tunnel where the drainage water is collected.

The tunnel is made of reinforced concrete; there are glass lights in the roof similar to those for lighting cellars under city sidewalks. Double doors to the entrance of the tunnel at the bottom of the steps and doors again at the top of the steps make the compartment secure against freezing.

Drainage water is collected in receptacles made of common sheet iron and lined with "Bakelite," a synthetic compound of phenol and formaldehyde. This substance forms an insoluble and chemically resistant enamel lending itself admirably to the purpose. It is baked on in three successive coats each at a temperature of about 100° C. for one to three hours. The receptacles are about 11½ inches in diameter and 2½ feet in height and are made uniform in size so that the volume of water can be measured by running a graduated glass rod to the bottom. Three cans are provided for each tank, one to receive the overflow in times of emergency and one for aliquot samples. The drainage comes at very irregular intervals, corresponding to prolonged periods of excessive rainfall. In the summer the rapid evaporation of water from the growing crop and from the soil surface seldom permits of 4 feet of soil becoming saturated.

In filling the tanks attempt was made, of course, to place the soil in very much the same condition as found in the field. The soil was taken up in three layers, 0–8″, 8–16″, 16–36″. The last depth formed the first two feet in the bottom of each 4-foot tank, the second depth occupied the next 12 inches in the tanks and the surface

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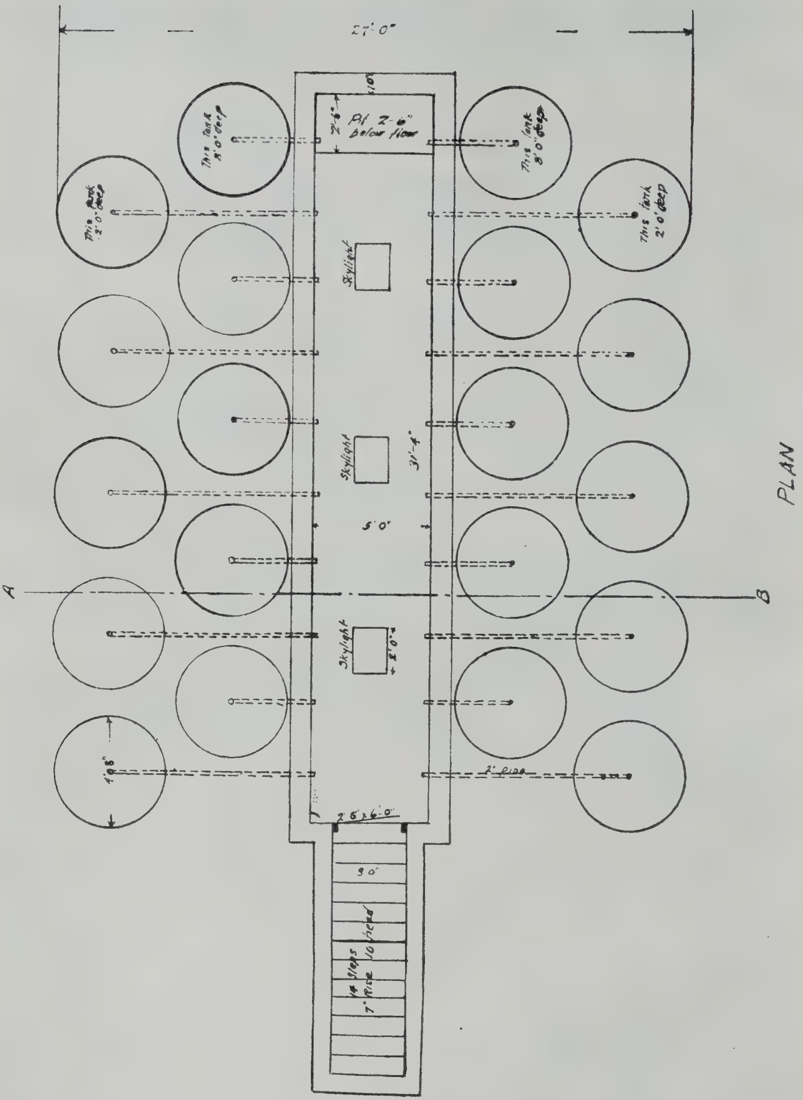
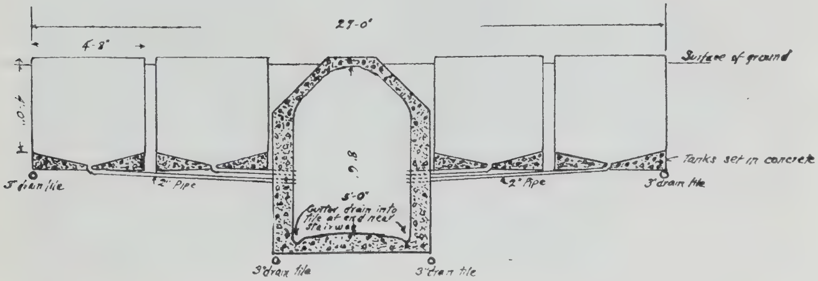
* Reprint of Technical Bulletin No. 61, March, 1917.


27'-0"
10"
2'-6"
Pit 2'-6" below floor
This tank 8'0" deep
This tank 8'0" deep
This tank 2'0" deep
This tank 2'0" deep
Skylight
Skylight
Skylight
31'-4"
5'0"
2'0"
A
B
4'8"
2" pipe
2'6"x6'0"
3'0"
14 Steps
7" Rise 10" tread
PLAN

BATTERY OF LYSIMETERS
FOR THE
NEW YORK AGRICULTURAL EXPERIMENT STATION
GENEVA NEW YORK.
DESIGN BY J. F. BARKER.



SECTION ON A-B

8 inches from the field formed about the surface 9 inches in the tanks, they being filled to only about 3 inches of the top. Owing to the large amount of stone below 36 inches it seemed impracticable to take the soil to the full depth of 4 feet and yet get it from the places desired, and it is mainly of importance to have the soil in the different tanks uniform rather than to duplicate exactly the condition at some particular place in the field. Especial attention was given to mixing each layer of soil before it was placed in the tanks. Eight tanks were filled with a soil of the type common to the hill lands of southern New York, very deficient in lime and light in color, and in texture a loam containing a small amount of sandstone shale. The twelve remaining tanks received soil from the Station farm, calcareous in nature, of reddish brown color and in texture a loam. The latter soil is naturally adapted to alfalfa and clover while the first named type is just the opposite in this respect. The weight of soil for each 4-foot tank was 7170 pounds for the first type and 6850 pounds for the second. The greater weight of the first was due mainly to the presence of more stone. The total cost of constructing this plant, including a woven wire fence for protection, was not far from $2200.

PROBLEMS FOR STUDY.

The main problem for study of which the tanks were designed has to do with the nitrogen balance in the soil as affected by legumes such as clover and alfalfa on the one hand and by non-legumes such

such as grasses and small grain on the other. The problem is one which has interested Director Jordan for a number of years and which he expressed a desire to have studied. Other problems include: (1) The effect of depth of soil upon the supply of moisture or available plant food upon the amount and composition of drainage water; and (2) the rate of loss of fertilizing constituents from the soil in general and as effected by fertilizers applied, difference in character of soil and kind of crop grown. Opportunities will no doubt arise in the course of the work to obtain side lights on additional problems.

The only excuse, of course, for an elaborate construction of this type is to provide means for collecting the drainage water from definite amounts of soil, and problems to be taken up in this connection are only those which require an examination of the drainage water as an essential part of the study.

The main purpose of having the two distinctly different types of soil — one naturally adapted to clovers and alfalfa, the other quite the opposite — is to study their effects upon the accumulation of nitrogen in the soil. In order to grow legumes successfully on the poorer soil it was necessary to apply a certain amount of lime. All tanks receive a liberal ration of phosphorus and potassium, since for the purpose of this study none of the crops should be limited by a deficiency of those elements. Tanks 7 and 8 receive nitrogen as dried blood applied to the alfalfa crops in amounts equivalent to the alfalfa produced on 1 and 2.

The following is an outline of the cropping system on the basis of a 4-year rotation for each tank.

		1915.	1916.	1917.	1918.
High lime requirement soil.	Lys. No. 1......	Alfalfa	Alfalfa	Barley	Wheat
	“ “ 2......	“	“	“	“
	“ “ 3......	Timothy	Timothy	Barley	Wheat
	“ “ 4......	“	“	“	“
	“ “ 5......	Alfalfa	Alfalfa	Fallow	Fallow
	“ “ 6......	“	“	“	“
	“ “ 7......	Alfalfa	Alfalfa	Barley	Wheat
	“ “ 8......	“	“	“	“
Calcareous soil.	“ “ 9......	Alfalfa	Alfalfa	Barley	Wheat
	“ “ 10......	“	“	“	“
	“ “ 11......	Timothy	Timothy	Barley	Wheat
	“ “ 12......	“	“	“	“
	“ “ 13......	Barley	Wheat	Alfalfa	Alfalfa
	“ “ 14......	“	“	“	“
	“ “ 15......	Alsike Clover,	Al. Cl.	Barley	Wheat
	“ “ 16......	“ “	“	“	“
	“ “ 17......	“ “	“	“	“
2 feet deep	“ “ 18......	“ “	“	“	“
	“ “ 19......	“ “	“	“	“
8 feet deep	“ “ 20......	“ “	“	“	“
	“ “ 21......	“ “	“	“	“

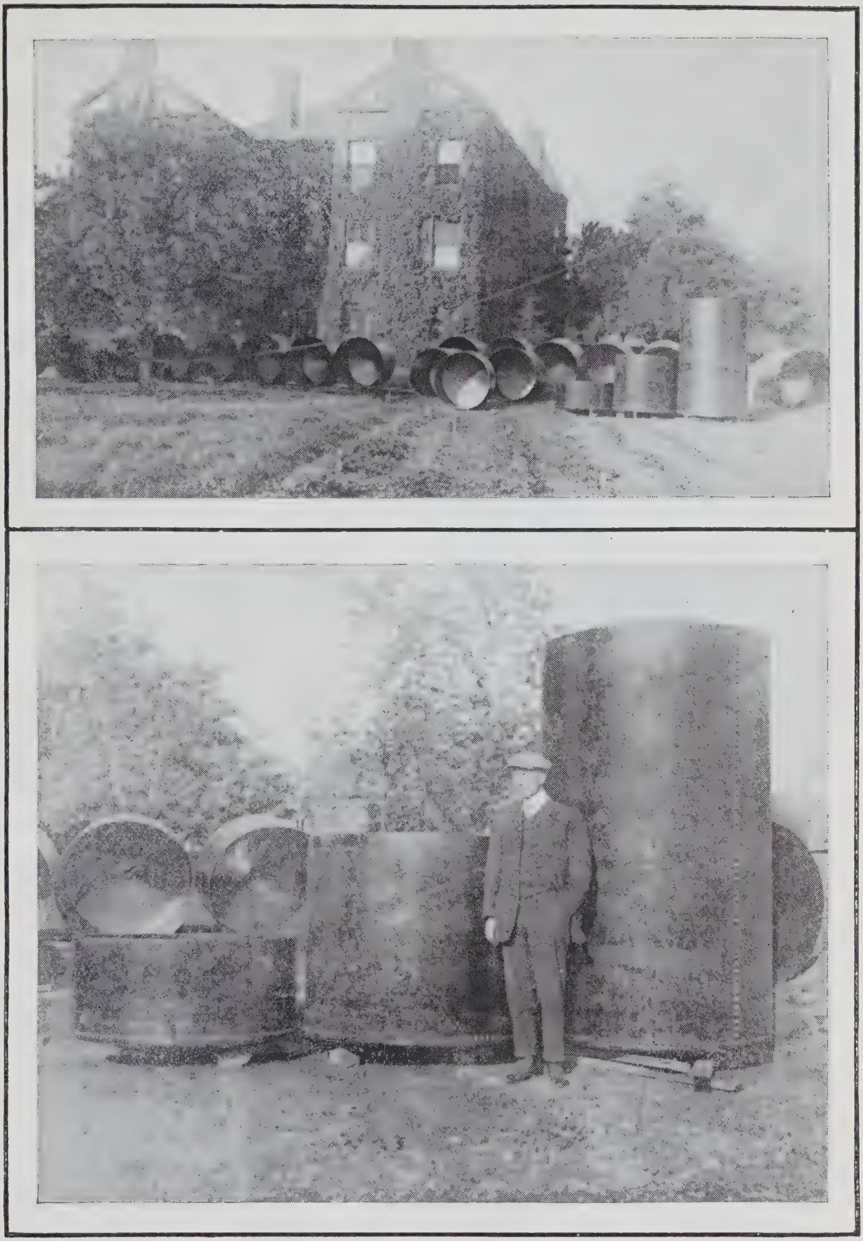


Plate V.

Upper.— Twenty Steel Tanks Assembled for Lysimeter Construction.

Lower.— Two-foot, Four-foot and Eight-foot Tanks.

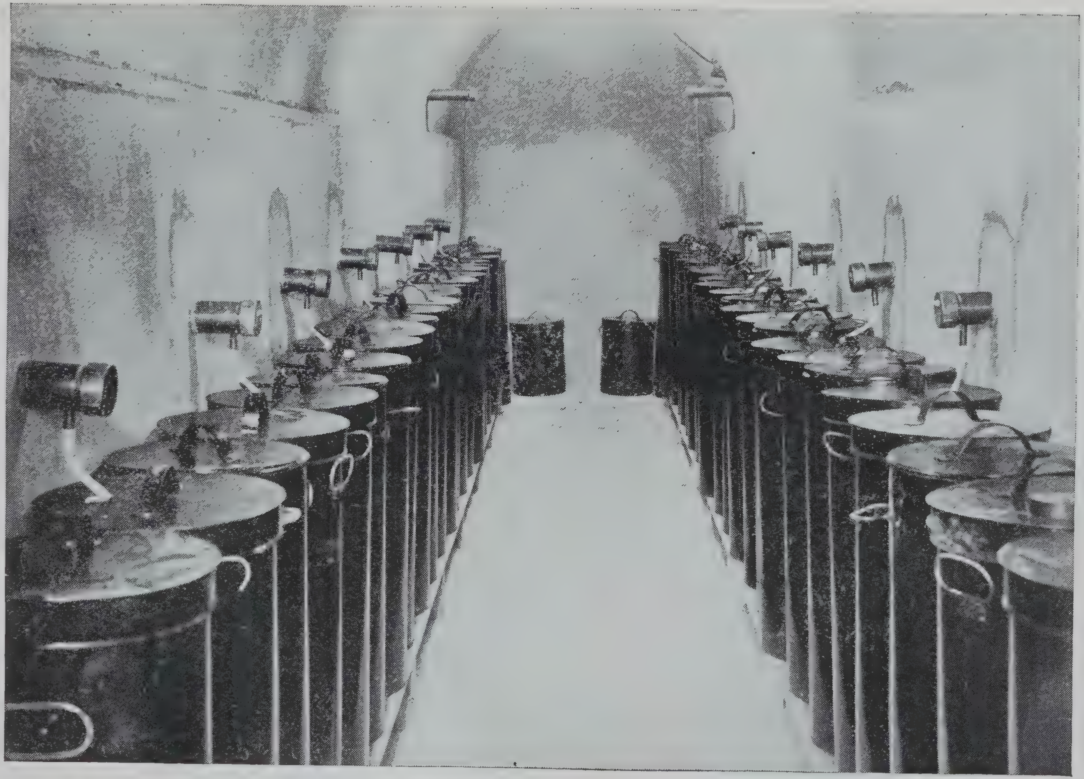


Plate VI.— Interior of Lysimeter Tunnel Showing Facilities for Collecting Drainage Water.

The chemical work required in connection with such a set of lysimeters demands a large part of one man's time.

When the tanks are first filled duplicate samples of the thoroly mixed soils are taken for each depth and for each of the two types; and complete analyses are made of these samples.

All fertilizers applied are sampled and analyzed.

Annual samples of the crops grown on each tank are analyzed for nitrogen, phosphorus, potassium, calcium, magnesium, sodium and total ash.

As the drainage water fills the receiving cans aliquot portions are saved and the remainder discarded. These aliquots are analyzed twice annually for phosphorus, potassium, sodium, calcium, magnesium, chlorine, sulphur, carbonates, bicarbonates, nitrates, nitrites and ammonia. In addition nitrates are determined every 2 to 4 weeks when tanks are flowing.

Samples of rain and snow water are examined for nitrogen.

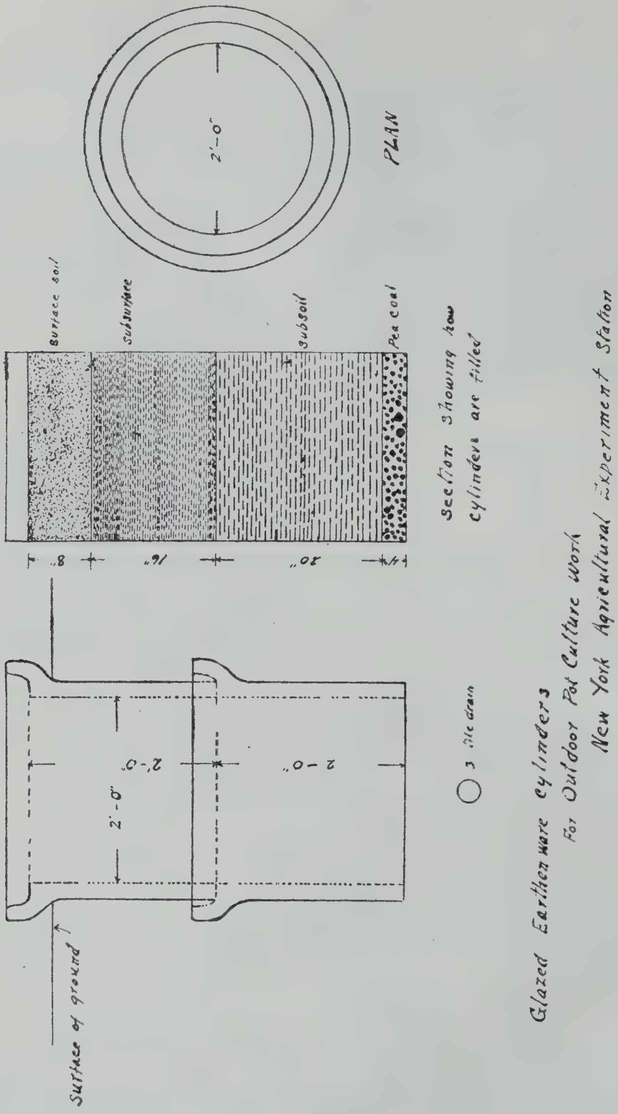
CYLINDERS FOR OUT-DOOR POT CULTURE WORK.

When fertility experiments are conducted on soils under greenhouse conditions a much larger percentage of experimental error must be allowed for than under well-planned field conditions. This is of course partly due to the very small quantities of soil used and consequently small amounts of crop grown under glass and also to the very artificial conditions imposed. Indeed, most common field crops can only with difficulty be brought to maturity under these conditions. Altho it would seem an opportunity to control accurately edaphic conditions, yet greater variations as regards temperature, moisture and sunlight are likely to occur at different places on the bench than on different plats in a carefully conducted field experiment. Also, soils having well known differences in productiveness in the field often show quite a different order of productiveness in the greenhouse.

The main advantage sought after in conducting fertility experiments under glass are: (1) Opportunity to make a large number of tests with little time and labor, (2) a chance to bring together under the same conditions different soil types, (3) avoiding the extremes of climatic conditions, (4) facilities for growing crops in the winter time, (5) means of controlling the exact amount and kind of plant food in the nutrient medium.

Up to date facilities for plant nutrition work arrange for having the plants out of doors all the time excepting at night and in inclement weather. As a step in advance of this many have tried having the pots or cylinders sunk in the ground and providing a movable covering.

After some experience with greenhouse experiments and after considering many methods used in pot-culture work, the author



Glazed Earthenware cylinders
For Outdoor Pot Culture Work
New York Agricultural Experiment Station

has devised facilities for outdoor pot work as described herewith. They are intended to place the experiments under nearly natural conditions, yet allowing the use of definite amounts and types of soil, with soil composition and fertilizer treatment accurately known. When growing perennial plants, as desired in these experiments, it is necessary that seasonal changes operate as usual.

CONSTRUCTION OF OUT-DOOR CYLINDERS.

The accompanying drawing shows the construction of these cylinders without much need of explanation. Two glazed earthenware sewer pipes each 2 feet long and 3 feet inside diameter are sunk in the ground to within a few inches of the top, making a cylinder 4 feet in depth and holding, as filled, approximately 1400 pounds of silt loam soil. A trench is first dug about ten inches lower than the bottom of the cylinders and in this is laid a line of 3-inch drain tile, thus completely preventing the rise of ground water into the cylinders. To isolate the soil in the pot from the influence of the surrounding soil it is necessary also to break capillary connections and this is accomplished by a 4-inch layer of some coarse material. We have used hard coal of " pea " size, all gravel in this section being loaded with lime carbonates.

When the cylinders are filled the soil is placed in layers corresponding to the surface, subsurface, and subsoil in the field. The right amount of soil for a given set of cylinders is assembled on a barn floor, a separate pile for each stratum. Each pile is then shoveled over until there is no doubt of its uniform composition. Samples are taken for analyses and it is transferred to the cylinders. To give opportunity for settling over winter, the work should be done in the fall. The soil is only lightly tamped and the cylinders are filled to the top. Before treatment is begun the next spring the surface is smoothed to a level with the shoulder of the cylinder leaving a basin 3 inches deep to catch rain water and prevent loss of soil. Fertilizer applications are incorporated with the surface 4-inches, that amount of soil being transferred to a large receptacle for the mixing.

The bell-shaped top of the cylinder allows about 50 per ct. more rainfall than is normal for the surface of the soil exposed. And this is an important advantage considering the break in capillarity and also that on separated small areas of soil larger crops can be grown than is normal to the same area in a crowded field. Lack of sufficient moisture should not be a limiting factor in this work and so it is advisable at times to water the pots by hand. For this purpose there should be a reservoir of rain water near by with piping to the vicinity of the pots. This precaution applies also to the lysimeters.

CYLINDERS IN OPERATION.

The first set of these cylinders were constructed at this Station in the fall of 1914. Another set were prepared in November, 1915, making a total of 98, each 4 feet deep. Of these 48 are given over to a fertility study with peach trees, and 50 others are used in lime requirement studies, alfalfa being grown on one series and rape being the first crop on another series. The soils used in these cylinders are of two types, the same as those used in the lysimeters, and have been taken from the same localities. All but 18 of the cylinders are filled with the southern New York soil high in lime requirement.

DETERMINATION OF CARBONATES IN LIMESTONE AND OTHER MATERIALS.*

J. F. BARKER.

SUMMARY.

This bulletin describes and illustrates a simple device for the determination of carbonates, particularly in limestones and similar materials. It is based on the principle of the hydrometer, requires no weighing and gives results without computations. From its inexpensiveness, simplicity and accuracy it should serve a useful purpose in a wide field.

RELIABILITY AND PRINCIPLE OF APPARATUS.

Results obtained in the determination of carbonates by the apparatus herein described compare favorably with results from the use of any standard laboratory method. The advantages of this method over others are that no chemical balance or scale is required and that there are no long calculations to be made. Anyone with some aptitude for accuracy can, with this instrument, make as reliable a determination in home or office as a skilled chemist in a well equipped laboratory using complicated apparatus.

The invention depends upon the principle of the hydrometer, which takes account of the law that when an object is immersed in a liquid it is buoyed up by a force equal to the weight of the liquid displaced by the object. In the apparatus the carbon dioxide gas set free from the sample decreases the weight; and the rise of the graduated scale tube above the water records the percentage of carbonates from which the gas was released.

PROCEDURE.

To analyze a sample of limestone for carbonates: Measure out 40 c.c. of HCl (sp. gr. 1.15) using a small graduate; pour this into the acid reservoir through the opening at A. With graduated stem disconnected hang 10-gram weight at B. The hydrometer should then float in a cylinder of water and be immersed to some point at C. Remove 10-gram weight and introduce pulverized limestone until instrument is immersed to exactly the same point. Now connect graduated stem and add water, a drop at a time, through

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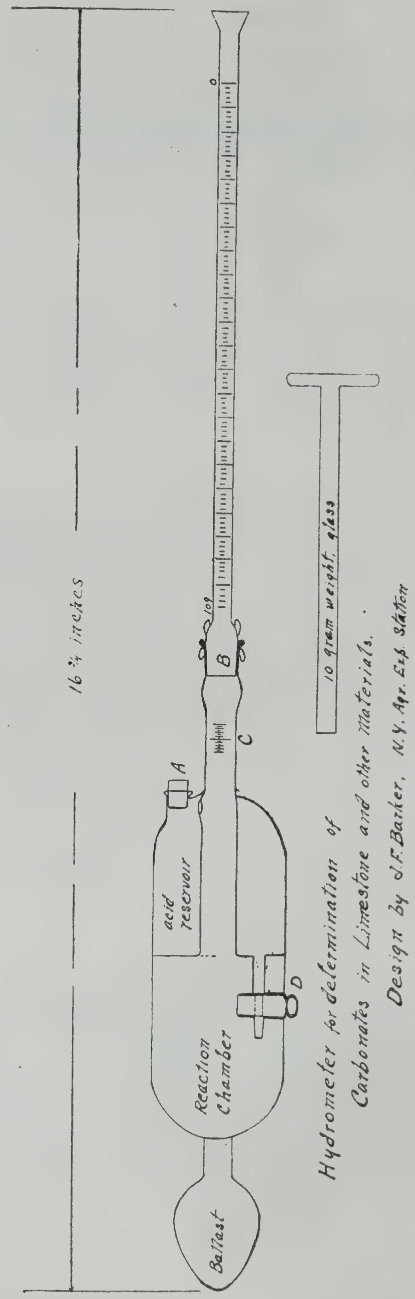
* Reprint of Technical Bulletin No. 62, May, 1917.



Hydrometer for determination of Carbonates in Limestone and other Materials.

Design by J.F. Barker, N.Y. Agr. Exp. Station the funnel-shaped top, until immersed to zero point. Raise hydrometer out of water and open stop-cock D until acid drops slowly into reaction chamber, decomposing the limestone; then release so apparatus floats freely. As the reaction proceeds the instrument slowly rises and at the conclusion the point on stem at surface of water gives the percentage of calcium carbonate equivalent to the carbon dioxide in the sample. This figure is the *calcium carbonate equivalent* so often mentioned in connection with limestone analyses.

A Fahrenheit thermometer accompanies each instrument and is hung inside the floating cylinder. Its reading is taken before and after each determination to allow for any error due to change in temperature. To the figure for calcium carbonate equivalent add 0.5 for each degree rise or subtract 0.5 for each degree fall in temperature between the two readings. This temperature change need seldom amount to more than a fraction of a degree.

The right amount of acid to be used is the amount that will cause the hydrometer with 10-gram weight to sink somewhere along the graduated space at C. This should be approximately 40 c.c. Some limestones or other materials will froth badly and in such cases the acid must drop very slowly.

DEGREE OF ACCURACY.

The accuracy and practicability of the method are well illustrated by the following results of analyses of limestone. The figures for this method were obtained by a farm boy without chemical training or special aptitude for such work. They do not simply represent a few of his best determinations, but are all he had made up to date. The other column gives results on the same samples obtained by a chemist using a standard laboratory method.

Limestone No.	Calcium Carbonate Equivalent.		Limestone No.	Calcium Carbonate Equivalent.	
	Hydrometer method.	Standard method.		Hydrometer method.	Standard method.
200	80.0	80.0	230	83.5	83.2
201	81.5	81.7	240	99.5	99
220	78.5	78.6	244	98.5	98.5
221	71.0	71.1	254	97.5	97.7
223	78.5	78.7	256	89.0	88.9
224	83.0	84.0	257	95.5	95.8
226	82.5	82.9	Calcite	99.5	99.8

SOME DETAILS.

For those who care to examine the method critically it should be said that sources of error have been eliminated until the method is accurate to the degree that the graduated stem can be read. This means about ¼ of one per ct. The limestone can be weighed to an accuracy of .02 gram. The weight of CO_2 remaining in the apparatus tends to offset the loss due to moisture escaping with the gas, but the difference, together with any other important sources of error, has been accounted for in the graduation of the reading stem. This graduation has been verified by the author, checking against samples of calcite, and any necessary corrections made so that the instrument as now obtainable gives readings which need no corrections save for changes in temperature occurring during the progress of a determination.

Each outfit of this apparatus includes a glass cylinder for floating the hydrometer, a small Fahrenheit thermometer, 500 c.c. bottle of hydrochloric acid (sp. gr. 1.15), 50 c.c. graduate, small dropping pipette and scoop for convenience in transferring the sample. The manufacturer's price for a single outfit, it seems now, will be less than ten dollars. Hydrochloric acid of the given strength can be obtained at any time from a local drugstore.

COMPOSITION OF LIMESTONE.

For the benfit of those who are not chemists it should be explained that limestones are composed of calcium carbonate, or calcium and magnesium carbonates, and a varying percentage of clay-like material. In good grades of limestone the carbonates make up 85 to nearly 100 per ct. of the stone. Calcium carbonate ($CaCO_3$) is composed of 40 parts calcium, 12 parts carbon and 48 parts oxygen, by weight. When calcium carbonate is treated with acid, as in this determination, all of the carbon and 32 parts of the oxygen pass off in the form of carbon dioxide gas (CO_2), and this 100 parts of calcium carbonate loses 44 parts as gas.

Magnesium carbonate ($MgCO_3$) is made up of 24 parts magnesium, 12 parts carbon and 48 parts oxygen, by weight. When treated with acid, carbon dioxide again passes off and 84 parts of the carbonate loses 44 parts as gas.

Now 24 parts, by weight, of magnesium may be said to be equivalent to 40 parts of calcium; for the two elements in these proportions neutralize the same amount of acid, either in the laboratory or in the soil. With this in mind a rereading of the above statements will make clear the fact that equivalent amounts of calcium and magnesium carbonates give off the same amount of gas.

In limestone analyses, then, the weight of gas given off is 44 per ct. of the weight of calcium carbonate equivalent to the total carbonates in the sample. With this fact in mind the stem of our hydrometer has been so graduated that with any given loss of carbon dioxide the figure for calcium carbonate equivalent is read directly.

Limestones having a high percentage of magnesium carbonate are decomposed by acid more slowly than others and this fact will usually enable a person making this determination to recognize such stones.

APPLICATION OF THIS METHOD.

For analyzing carbonates other than limestone and similar materials, this instrument is fitted with a graduated stem which reads percentage of carbon dioxide; an arrangement which greatly extends its use. It will be found especially suited to determining the comparative strength of baking powders, more suitable, considering speed and accuracy, than any other device now employed for that purpose.

The method is adapted for use in all chemical laboratories: college, experiment station, or commercial laboratories. However, it is devised for use outside of chemical laboratories, also. Limestone companies can employ it to check up on every carload of their goods, or to locate the stone in their quarry most suitable for grinding. County agricultural agents will use it to keep posted on the quality of limestone being sold in their territory and to determine the composition of local limestone deposits. Many an individual farmer or some member of his family will no doubt find it a source of satisfaction to own this outfit with the object of examining purchases of limestone or other carbonates.

REPORT

OF THE

Department of Animal Industry.

W. H. JORDAN, *Director.*
G. A. SMITH, *Dairy Expert.*

TABLE OF CONTENTS.

I. Goat's milk for infant feeding.
II. Milking machines.

REPORT OF THE DEPARTMENT OF ANIMAL INDUSTRY.

GOAT'S MILK FOR INFANT FEEDING.*

W. H. JORDAN AND G. A. SMITH.

SUMMARY.

1. During the years 1910–1912, inclusive, a herd of milch goats was kept at the Station. The number of animals of which complete records were kept varied from 10 to 26.

2. In the year 1912, 31 adult and 9 partially grown animals were fed. The quantity of food consumed was as follows:

Dry, coarse food	37,740	lbs.
Beets	1,550	"
Cut grass	24,000	"
Pasture	132	days
Grain	14,688	lbs.

3. The total cost of this food, at the prices then ruling, was $441.95. The average cost per month per goat varied from $0.481 to $0.992. The average cost of food per goat per year was $11.05, making the daily cost $0.03.

4. The yearly production of milk, including some animals in the first period of lactation, varied from 301.7 pounds to 1,845.2 pounds. The average yearly yield for 10 animals of which records were kept during 3 years, including 28 lactation periods, was 800.4 pounds.

5. The food cost of the milk per goat for all the goats during the year 1912 was 4 cents per quart and for the three years during which the record was kept 3.4 cents. The lowest cost was with the Saanen goat, No. 11, for the year 1911, which was estimated to be 1.27 cents per quart. The other items of cost, such as care and overhead charges, it is not possible to give with any accuracy. The average food cost for a quart of milk from the Station herd of 25 Jerseys during the three years has been found to be .92 cent per quart.

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* Reprint of Bulletin No. 429, February, 1917.

6. The range of composition of the mixed milk of the whole flock as determined during May and June of the year 1912 was as follows: Solids, 11.4 per ct. to 11.9 per ct.; solids not fat, 7.72 per ct. to 8.61 per ct.; fat, 3.5 per ct. to 3.8 per ct.

The composition of milk from individual goats was found to vary in total solids from 9.22 per ct. to 18.55 per ct.; in protein, from 2.24 per ct. to 4.96 per ct.; in casein, from 1.56 per ct. to 4.6 per ct.; in fat, from 1.08 per ct. to 8.4 per ct.; and in ash, from 0.43 per ct. to 0.8 per ct.

7. A chemical study of the goat's milk indicated no essential difference between the constitution of its casein and that of cow's milk. Marked and probably important differences were observed in the salts of the ash as compared with the ash of both cow's milk and human milk.

8. Extensive study of the use of goat's milk in infant feeding by Doctors Sherman and Lohnes, of Buffalo, showed that the curds of goat's milk when returned from the stomach were smaller and more flocculent than those of cow's milk. From the determination of the combined hydrochloric acid in the returned food, the authors conclude that the cow's milk had a greater stimulating effect on the stomach than goat's milk. The absorption of the food and gain in weight in comparing the two milks were indefinite for several reasons. The babies tolerated equally well similar amounts of goat's milk and cow's milk when used with the same diluents. The younger the child, the more the evidence pointed toward a greater gain on goat's milk.

9. Goat's milk was supplied to 18 cases of children that were not thriving on any other food that had been tried. In 17 cases a satisfactory state of nutrition was established through the use of goat's milk, the beneficial results in some instances being very marked. With certain of these children their situation was regarded as serious, and their restoration to a satisfactory nutritional condition was good evidence that goat's milk is often a very desirable resort for infant feeding.

[The Station herd of goats has been sold, so no milk is now available for any purpose.]

EXPERIMENTAL ANIMALS.

The value and use of the milch goat have received considerable attention during the past few years. Very few data seem to have been available concerning this animal. In view of this fact, the Board of Control of this institution, some eight or ten years ago, authorized the purchase and importation of not over six high-grade Swiss goats, of either the Saanen or Toggenberg breed. A quarantine, established because of outbreaks of foot-and-mouth disease on the Continent and in England, prevented carrying out this plan. Some two or three years later Mr. H. S. Greims, of New York City, offered to present to the Experiment Station a flock of goats then in his possession. The offer was accepted, and Mr. Greims very generously shipped the animals to the Station, with considerable apparatus, without charge to the institution. We understand that the flock was purchased from a Mr. Riddle, of New Jersey. As the venture had not proved entirely satisfactory to Mr. Greims, due to the loss of some of his best does and because of his difficulty in having them cared for and their records kept as he desired, he concluded to abandon the enterprise.

The flock included some very good animals, one full blood Saanen and several full blood Toggenbergs, but many of the other animals were of inferior value and of no especial breeding. Some of these were promptly discarded. The better animals were kept, and an attempt was made during several years to ascertain the amount of food consumed and the quantity and composition of the milk produced.

When the goats reached us on February 25, 1910, they were in poor condition. Several were very thin in flesh and did not respond to feed and care. No food combination that we could offer appealed to their appetites. A veterinarian who was called in for advice suggested no treatment that was of benefit. The affected animals gradually grew weaker and died. Finally two of the dead goats were sent to Dr. V. A. Moore, Dean of the State Veterinary College, Ithaca, N. Y. After a careful study of the case, the disease was pronounced to be Takosis. This disease had been studied by the Department of Animal Industry, at Washington, and a bulletin published by that Department (No. 45) gives a very complete history of several outbreaks of the trouble. Our experience agreed with the facts as brought out in that bulletin. By placing the animals in a light, well ventilated stable, with sanitary surroundings, we were able gradually to get rid of the disease, and after 1911 did not have any more cases and have seen no signs of the trouble since.

There were received by us forty-nine goats, old and young, with two males, but owing to the conditions described we were not able to secure very many data in 1910. Only nine does were in condition to give milk that year. In 1911 conditions were much improved, as only two cases of Takosis developed, both in the spring. Early in 1912 all the animals were apparently normal, and of the twenty-eight old enough to breed twenty-three goats gave milk.

Cost of maintenance.— There were kept at the Station during the year beginning January 15, 1912, and ending January 14, 1913, twenty–eight adult females, three males, and nine kids that were dropped in 1911. It was not practicable with the number of goats that were kept together, and because of their uncontrollable, wasteful habit of eating, to keep a separate account of the amount of food consumed by individual animals. The whole amount for one day's feed was weighed and given the herd in two feeds, night and morning. As nearly as could be ascertained there was very little difference in the individual consumption of coarse food. About one pound of grain was fed daily to each animal. The food charged to the goats during one year was as follows:

TABLE I.— AMOUNT AND COST OF FEED FOR MILCH GOATS.

(Adult males, 3; adult females, 28; kids, 9.)

FOR YEAR 1912.

	Days fed.	Amount used.	Cost per 100 lbs.	Total cost.
		Lbs.		
Grain	365	14,688	$1.45	$212.96
Bean pods	199	18,180	.35	63.63
Beets, mangel	46	1,550	.20	3.11
Hay, mixed	273	19,560	.50	97.80
Grass	122	24,300	.15	36.45
Pasture	132	....	....	28.00
Total	....	....	....	$441.95

COST OF FEED BY MONTHS.

1912			Average per goat
January	17 days	$19.25	.481
February	29 “	32.64	.816
March	31 “	34.93	.873
April	30 “	36.14	.904
May	31 “	33.68	.842
June	30 “	31.64	.791
July	31 “	38.30	.957
August	31 “	36.95	.924
September	30 “	39.42	.985
October	31 “	39.70	.992
November	30 “	38.62	.965
December	31 “	39.70	.992
January, 1913, 14 days		20.98	.524

Yield of milk.— For three years an accurate record was kept of the individual milk production of the animals we retained, together with certain animals that were bred after the goats were received. The record of total yearly yields follows:

Table II.— Milk Yield of Milch Goats.

	Milk Yield.		
	1910 *Lbs.*	1911 *Lbs.*	1912 *Lbs.*
No. 5. Full blood Schwartzenberg	678.3	913.3	600.7
No. 6. “ “ Short Haired Toggenberg	778.4	1,455.7	377.1
No. 7. “ “ “ “ “	186.5	763.5	870.7
No. 8. “ “ “ “ “	1,189.	838.3	*
No. 9. “ “ Long “ “	210.3	1,167.1	705.5
No. 10. “ “ Short “ “	†	1,361.4	869.2
No. 11. “ “ Saanen	1,028.5	1,845.2	1,391.1
No. 12. “ “ Schwartzenberg	120.5	613.	424.
No. 15. Toggenberg-Angora	496.5	801.3	725.5
No. 16. “ “	457.	889.4	655.9
No. 17. “ “		211.	63.9
1910 kid of No. 5		846.	861.
Toggenberg-American		406.5	472.
1911 kid of No. 5			741.2
1911 kid of No. 5’s 1910 kid			‡
1911 “ “ “ 6			428.1
1911 “ “ “ 10			528.1
1911 “ “ “ 12			583.2
1911 “ “ “ 16			336.8
Schwartzenberg-Toggenberg			409.9
Toggenberg-Saanen			439.1
“ “			413.7
“ American			489.6
“ “			518.8
Saanen-Angora			301.7

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* Would not breed. † Did not breed. ‡ No milk.

It is to be noted that during 1911, the production of four of these animals, Nos. 6, 9, 10 and 11, was very satisfactory. With only one animal did this standard of production continue during 1912, namely, No. 11. This pure bred Saanen appears to have been a somewhat unusual animal, as her milk yield in 1911 was 1845.2 pounds. During 1911 and 1912 several of the animals gave from 700 to 900 pounds of milk, which is perhaps as good a yield as may be expected, excepting from animals considerably above the average. It should be noted that the period of lactation is considerably shorter than with cows, continuing from 250 to 300 days with most of the does.

The cost of food and milk.— The average cost of food per goat for the year was $11.05, making the cost per day three cents. If it was possible to get any number of goats together like No. 11, the Saanen, the keeping of goats would be a profitable proposition for a family supply. On the basis of a food cost of eleven dollars a year, her milk, during the year of best production, would cost 1.27 cents a quart, the average for the three years being 1.65 cents a quart. The best Toggenberg one year produced milk for 1.62 cents a quart, but her average for the three years was 2.70 cents a quart. The average food cost of the milk, for 1912, of all the goats was 4 cents a quart, and the average cost for the three years was 3.4 cents a quart. The average fat content of the milk was 3.71 per ct., of total solids 11.76 per ct. The average food cost of a quart of milk with the Station herd of 25 Jerseys during the past three years has been .92 cent per quart; the average fat content 5.9 per ct., and total solids 15.20 per ct. This shows the cows to be cheaper producers of milk and milk solids than the goats under the conditions prevailing at this Station.

Composition of the mixed milk.— During May and June of the year 1912 the mixed milk of the herd was analyzed daily. This was done by the use of the lactometer and the Babcock test. Comparison of this method with the gravimetric method showed it to have the necessary accuracy. The results follow:

Composition of Mixed Milk of Goats.

	Lactometer.	Fat.	Solids not fat.	Total solids.
		Per ct.	*Per ct.*	*Per ct.*
May 1	31.0	4.3	8.61	12.91
2	30.5	4.4	8.50	12.90
3	29.6	4.4	8.28	12.68
4	29.5	4.0	8.17	12.17
5	30.0	4.0	8.30	12.30
6	29.5	3.9	8.15	12.05
7	29.5	3.8	8.13	11.93
8	29.5	3.8	8.13	11.93
9	29.0	4.0	8.05	12.05
10	29.0	3.8	8.01	11.81
11	29.5	3.7	8.11	11.81
12	28.5	3.6	7.84	11.44
13	28.5	3.8	7.86	11.66
14	29.2	3.8	8.06	11.86
15	29.5	3.8	8.13	11.93
16	29.0	3.9	8.03	11.93
17	29.0	3.9	8.03	11.93
18	29.0	3.8	8.01	11.81
19	28.5	3.7	7.86	11.56
20	29.0	3.9	8.03	11.93
21	29.0	3.8	8.01	11.81
22	29.0	3.8	8.01	11.81
23	28.5	3.6	7.84	11.44
24	28.5	3.7	7.86	11.56
25	28.8	3.6	7.92	11.52
26	28.0	3.6	7.72	11.32
27	29.0	3.7	7.99	11.69
28	28.5	3.9	7.90	11.80
29	28.5	3.6	7.84	11.44
30	29.0	3.7	7.99	11.69
31	29.5	3.8	8.13	11.93
June 1	29.5	3.8	8.13	11.93
2	29.0	3.5	7.95	11.45
3	29.0	3.6	7.97	11.57
4	28.5	3.6	7.84	11.44
5	29.5	3.7	8.11	11.81
6	29.5	3.4	8.05	11.45
7	29.6	3.4	8.08	11.48
8	30.0	3.6	8.22	11.82
9	30.0	3.5	8.20	11.70
10	30.0	3.4	8.18	11.58
11	29.5	3.5	8.07	11.57
12	30.3	3.3	8.23	11.53
13	30.0	3.2	8.14	11.34
14	30.2	3.4	8.23	11.63
15	30.0	3.6	8.22	11.82
16	30.0	3.5	8.20	11.70
17	29.2	3.4	7.98	11.38

Examination of these figures shows that the percentage of fat in the mixed milk varied from 3.2 per ct. to 4.4 per ct. As a rule, the fat percentage ranged between 3.5 per ct. and 3.8 per ct. The solids-not-fat ranged from 7.72 per ct. to 8.61 per ct., the general range being between 7.8 per ct. and 8.2 per ct. The total solids ranged in general between 11.4 per ct. and 11.9 per ct. Analyses of the milk from individual animals showed much greater variation. This is illustrated by the figures which follow:

TABLE III.— ANALYSES OF MILK FROM INDIVIDUAL GOATS.

NUMBER OF GOAT.	Date of milking.	Specific gravity.	Fat.	Total solids.	Proteins.	Casein.	Ash.
			Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
5	Aug. 2	1.0269	3.7	11.43	2.87	2.10	0.51
5	Aug. 4	1.0269	4.0	11.93	2.86	2.08	0.44
5	Aug. 9	1.0264	3.7	11.47	2.88	2.13	0.49
6	Aug. 2	1.0280	2.5	10.18	2.55	1.90	0.52
6	Aug. 4	1.0278	2.5	9.91	2.24	1.56	0.44
6	Aug. 9	1.0286	2.7	10.49	2.48	1.64	0.48
7	Aug. 2	1.0278	3.8	11.42	2.58	1.95	0.56
7	Aug. 4	1.0286	3.8	11.54	2.58	1.83	0.51
7	Aug. 9	1.0280	3.9	11.80	2.57	1.71	0.57
8	Aug. 2	1.0287	3.0	10.56	2.54	1.78	0.57
8	Aug. 4	1.0282	2.6	10.23	2.37	1.56	0.50
8	Aug. 9	1.0286	3.0	10.73	2.48	1.66	0.49
9	Aug. 2	1.0294	3.4	11.53	3.03	2.12	0.57
9	Aug. 4	1.0295	3.1	11.08	2.78	2.00	0.54
9	Aug. 9	1.0298	3.4	11.11	2.77	1.83	0.51
11	Aug. 2	1.0274	2.4	9.61	2.50	1.86	0.45
11	Aug. 4	1.0266	2.2	9.22	2.27	1.56	0.51
11	Aug. 9	1.0275	2.4	9.66	2.34	1.59	0.43
12	Aug. 2	1.0354	3.6	13.73	4.74	3.78	0.65
12	Aug. 4	1.0359	3.8	14.51	5.21	3.82	0.56
12	Aug. 9	1.0322	5.6	15.18	4.15	3.26	0.53
15	Aug. 2	1.0294	2.2	10.29	3.08	2.32	0.50
15	Aug. 4	1.0300	1.8	9.90	2.98	2.26	0.52
15	Aug. 9	1.0287	3.0	10.23	2.88	2.13	0.53
16	Aug. 2	1.0293	2.8	11.18	3.26	2.64	0.55
16	Aug. 4	1.0294	2.8	10.95	3.30	2.54	0.40
16	Aug. 9	1.0303	3.4	11.79	3.33	2.48	0.61
20	Aug. 2	1.0314	7.8	17.63	4.96	4.06	0.76
20	Aug. 4	1.0336	6.0	16.17	4.95	4.02	0.73
20	Aug. 9	1.0303	8.4	18.55	4.83	3.85	0.80
23	Aug. 2	1.0300	6.3	15.47	3.83	3.14	0.70
23	Aug. 4	1.0296	6.0	15.23	3.70	2.90	0.59
23	Aug. 9	1.0310	6.5	16.13	3.94	3.07	0.68
General average	......	1.0294	3.82	12.12	3.21	2.40	0.55

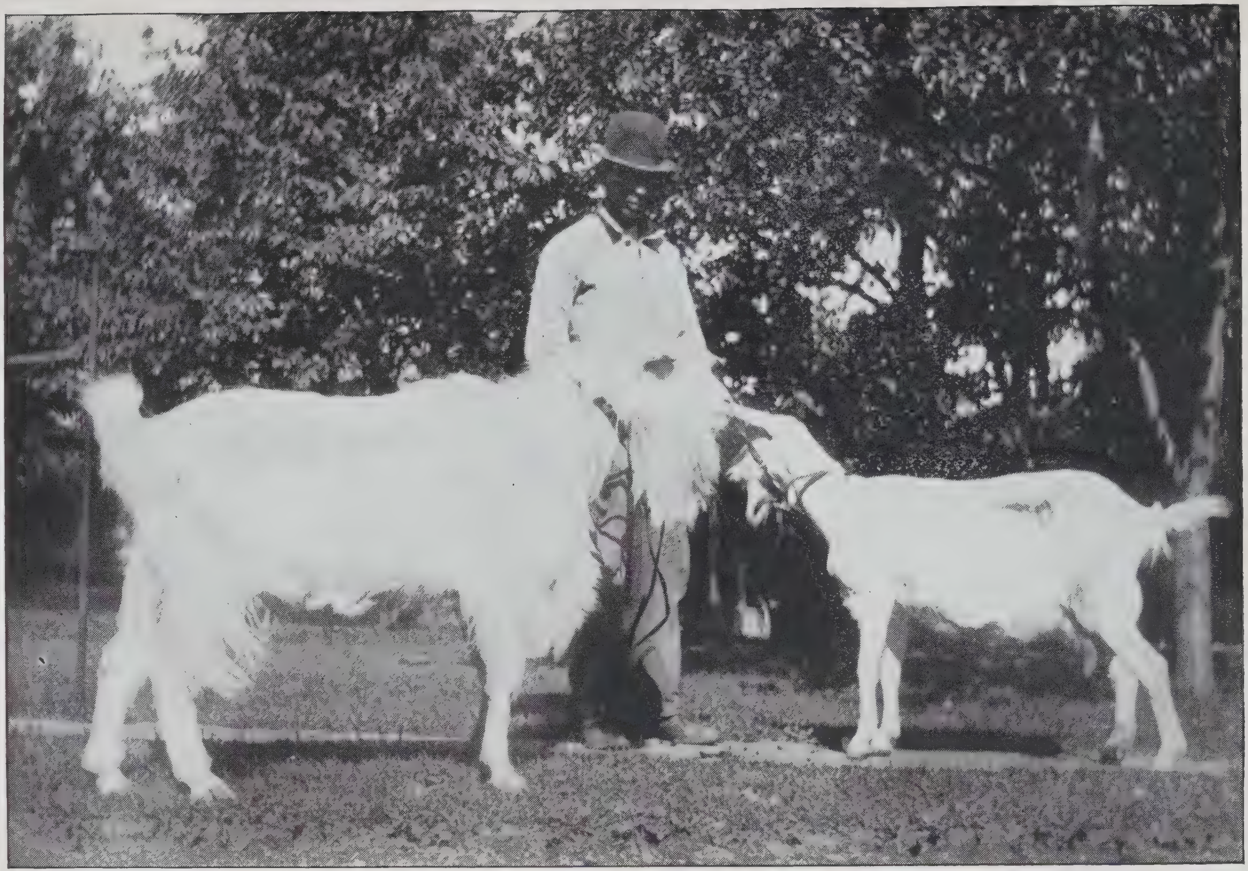

Plate VII.— Typical Buck and Doe of Saanen Milch Goat.

Doe is No. 11, of Station; buck property of C. L. Nicholls, Lockport, N. Y., to whose courtesy Station owes potograph from which plate was made.

Plate VIII.— Milking a Goat.

Milking should be done out doors or in room separated from stable, to avoid unpleasant odor in milk.

The data indicate a wide variation in composition. Animals Nos. 20 and 23 were well along in lactation.

A chemical study of goat's milk.— In 1915, Mr. A. W. Bosworth, at that time Associate Chemist at this institution, and L. L. Van Slyke, Chemist, made a somewhat elaborate chemical study of goat's milk in comparison with cow's milk and human milk. The results of this investigation may be seen in Technical Bulletin No. 46, issued by the Station in December, 1915, entitled " The Casein and Salts in Goat's Milk." It is not desirable to reproduce the text of that bulletin in this connection, but a few of the most important conclusions are here reviewed:

1. So far as could be judged from its combinations with bases, the casein of goat's milk does not differ essentially from that prepared from cow's milk.

2. Both goat's milk and cow's milk contain much larger percentages of solids than is the case with human milk.

3. Goat's milk contains practically the same amount of calcium phosphates as cow's milk, excepting that in goat's milk a certain proportion is in the tri-calcium form which is not true of cow's milk. In this respect both differ from human milk which appears to contain no calcium phosphates.

4. Cow's milk and goat's milk contain a much larger proportion of the magnesium phosphates than does human milk.

5. The proportion of mono-potassium phosphate was found to be practically the same in goat's milk and human milk. Cow's milk was found to contain di-potassium phosphate of which goat's milk and human milk were found to contain none.

6. The proportion of potassium citrate was found to be much larger in goat's milk than in either cow's milk or human milk; on the other hand, cow's milk was found to contain considerable sodium citrate, of which goat's milk contains none and human milk only a small proportion.

7. Goat's milk was found to contain both sodium and potassium chlorides, of which cow's milk and human milk contained none.

8. Calcium chloride was found in about equal proportion in cow's milk and goat's milk, being about twice as large in both cases as found in human milk.

As will be seen later, these differences in the composition of the three milks studied do not at present furnish an explanation of the reason why goat's milk appears to have been better adapted in many instances to infant feeding than cow's milk.

GOAT'S MILK AS FOOD FOR INFANTS AND VERY YOUNG CHILDREN.

Experience seems to have indicated that goat's milk has a peculiar adaptability to feeding infants and young children in cases where they must be given artificial food and neither modified cow's milk nor the proprietary infant foods are fed with success. Because of this fact, it was determined to make a somewhat exhaustive study of the applicability of goat's milk to infant feeding.

An effort was made to get data in two ways: (1) By a somewhat extensive series of observations carried on in cooperation with Doctors DeWitt H. Sherman and Harry R. Lohnes, of Buffalo; and (2) by supplying goat's milk for use with infants in those cases where other foods had been found inapplicable. The physicians mentioned carried on their study in the Saint Mary's Infant Asylum and Maternity Hospital, in Buffalo, to which institution practically 16 quarts of goat's milk was shipped daily in an iced container. This milk was drawn under the best of conditions and was found to be in good condition at the time of arriving at the hospital.

The results of this work have been published in the *Journal of the American Medical Association.*[1] The text of the report is here reproduced:

PRACTICAL STUDY OF GOAT'S MILK IN INFANT FEEDING AS COMPARED TO COW'S MILK.

DE WITT H. SHERMAN, M. D., AND HARRY R. LOHNES, M. D.
Buffalo, N. Y.

This study was made to learn, if possible, the reason why goat's milk agrees better with some infants than cow's milk.

The babies were selected at random from the inmates of St. Mary's Infant Asylum and Maternity Hospital.

The goat's milk averaged from .5 per ct. to 1.50 per ct. richer in fats than the cow's milk used in this institution. Nevertheless, in making our modifications of the two milks the same amount of either milk was used in the stock formulae. Consequently the caloric value of the goat's milk modifications were greater than of the cow's milk.

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[1] *Jl. Amer. Med. Assn.* 62:806, 807. 1914.

I will present our data under different headings, for example:

I. *Results of Gastric Analyses.*

All test meals were withdrawn one hour from the middle of the feeding. The *amount recovered* averaged, in fourteen cases, nearly twice as much of cow's milk formulae as goat's milk, the accurate ratio being 41 c.c. to 27 c.c. This points to the lower digestion of cow's milk.

The curds of the goat's milk formulae were smaller and more flocculent, corresponding to the appearance in test tube digestion.

The analyses in fifteen cases showed in goat's milk an average of:

Free hydrochloric acid	Once
Combined hydrochloric acid	22.00
Acid salts	5.53
Total acidity	28.4

The analyses in fourteen cases, the same infants, on *cow's milk* gave an average of:

Free hydrochloric acid	None
Combined hydrochloric acid	28.20
Acid salts	5.30
Total acidity	33.50

In a previous paper on " Gastric Analyses in Infants " we calculated the average gastric analysis on (a) barley water and (b) proprietary foods containing some proteids, fat, sugar and carbohydrates but no milk. For convenience of comparison we give below a table showing the averages of these and also of modified goat's and cow's milk, as shown above:

	Free hydrochloric acid.	Combined hydrochloric acid.	Acid salts.	Total acidity.
I. Barley water	2.10	5.60	....	9.00
II. Proprietary foods containing some proteids, fat, sugar and carbohydrates, but no milk	0.	10.50	....	17.40
III. Goat's milk with rice water and 2 per ct. cane sugar solution	0.	22.00	5.53	28.40
IV. Cow's milk with similar and other modifications	0.	28.20	5.30	33.50

This table indicates the greater stimulating effect on the stomach of cow's milk than goat's milk; the greater stimulating effect of both than of the proprietary foods made without milk; and finally the greater stimulating effect of all three than of barley water.

In some vomiting cases this table gives definite information as to the causal factor of this condition in these four kinds of foods — (a) through direct gastric stimulation, and (b) because of the size and density of curds, and (c) because of slower digestion.

As regards *absorption* and *gain* in weight our statistics are indefinite for three reasons. First, the babies being institution babies were apt to gain slowly: Second, owing to an epidemic of streptococcus infection which had swept through the infant's ward causing gastro-intestinal disturbances so serious that about one-fifth of the babies died, the digestion of those who did recover was more or less impaired; and third, because a certain number who were doing well were removed from the institution through adoption before our experiments were finished.

Of the number tested, sixteen cases in all, on similar formulae, twelve gained more rapidly on cow's milk modifications, and four on goat's milk. The gain of the former group was in the ratio of three to one. The gain of the latter group was in the ratio of nine to one. Consequently those who did gain on goat's milk gained more rapidly than on cow's milk, but fewer gained on goat's milk.

In *infantile atrophy and inanition*, three cases, goat's milk was no more suitable than any other food.

Taste of goat's milk.—At first the babies did not finish all their bottles, not liking the flavor of the food as well as of cow's milk. This surprised us because there seemed to us to be no unusual taste, as is usually supposed to be the case. If the milk is clean, we think the strong taste so common in Switzerland is unnecessary, and believe that the taste, as well as odor, is due to lack of udder cleanliness or possibly the type or food of the goat. But taken from three sources in this state — Geneva, East Aurora, and Buffalo — we have never found the strong taste or odor commonly supposed to be characteristic of goat's milk.

Fats.—In goat's milk the fat averaged from .5 per ct. to 1.50 per ct. higher than in cow's milk. The more thorough emulsification of the fat in goat's milk, possibly the finer fat globules, prevents the separation of the cream upon standing, as occurs in cow's milk. This fact may be an element in reducing the tendency to regurgitation in goat's milk as compared to cow's milk, and further, may be a very important factor in lessening the liability to sour vomiting due to fatty acid fermentation, so common in high fat mixtures of cow's milk.

Strength of formulae.—The babies, as a whole, tolerated equally well similar amounts of goat's or cow's milk with the same diluents, but as the goat's milk contained higher fat than cow's milk, they actually received more fat per feeding, and hence higher caloric value. It is consequently strange, that more babies gained, as stated above, on cow's milk than on goat's milk modifications.

In our formulae fat rarely averaged over 2¼ per ct. in goat's milk, and a little less than 2 per ct. in cow's milk.

Stools.—On goat's milk the stools were as a rule smaller and of a more vivid yellow color.

Age.—Age did not influence materially the ratio of gain on goat's versus cow's milk, but the younger the baby, the more the evidence pointed toward a greater gain on goat's milk.

Vomiting.—Of twenty-four children receiving both goat's and cow's milk formulae, five vomited goat's milk some, and none cow's milk. The explanation may be the slightly lower fat percentage in the cow's milk formulae. On the other hand, in certain cases goat's milk is often tolerated in similar amounts due to the fact that there is less gastric stimulation, that is, smaller, lighter curds.

After getting accustomed to goat's milk more children seemed hungry, and cried more at night than when taking cow's milk.

Type of modification commonest used was similar amounts of goats milk or cow's milk diluted with rice water and 2 per ct. cane sugar.

We are very much indebted to Doctor W. H. Jordan, director of the New York State Agricultural Experiment Station, for sending us for six weeks sixteen quarts a day of goat's milk in the best of condition. We are also indebted to Sister Frances and Sister Clare at St. Mary's Infant Asylum and Maternity Hospital for their assistance and cooperation in our work.

Practical results with individual children.— During several years the Station gave goat's milk without charge to all applicants, so far as the supply would permit, where it was desired to try this milk with infants or young children who were not thriving on any other kind of food. The only condition imposed was that reports should be made to the Experiment Station of the results of the experimental feeding. There follows the testimony of parents as to the outcome of the feeding and in a few instances the notes of attending physicians. It will be observed that this testimony is largely from parents, the professional judgment of physicians not being available in many of these cases. It is fair to conclude, however, that evidence so marked, even if given on an unprofessional basis, is not to be disregarded.

No. 1.— Mrs.———

I cannot thank you enough for the milk we are getting. Our baby has improved wonderfully on it. He has gained two pounds in the three weeks we have been using it. We tried everything we could find for him but nothing would agree with him until we got the goat's milk.

No. 2.— Mr.———

This milk has done more for our babe than any food we have used, as yet. Thanks to you for your kindness, I will report again at week end or according to your orders.

No. 3.— Mrs.———

I will give you as nearly as possible the results we had with the goat's milk. The baby had been troubled with constipation and with vomiting, showing large undigested curds. We started on the goat's milk, diluting it about one-fourth with water. She took to it kindly and conditions greatly improved. A little later I added between one and two tablespoons of cream to the goat's milk and that seemed to satisfy her better and still agreed with her. As warm weather come on, she turned against it, and I could not get her to take it any longer.

No. 4.— Mrs.———

We certainly appreciate your kindness in furnishing the milk and as you were interested in the results, I am delighted to say that our baby has gained in one month three and one-half pounds in weight, which is quite remarkable for her as for months her weight was the same, and she could not take the cow's milk.

No. 5.—

We began April 1st when the baby was 11½ months old feeding goat's milk as a supplement to mother's milk, giving 20 to 25 ounces a day. Prior to this time — was a healthy, breast-fed baby, normal, except that she was troubled with constipation. Weight 23 pounds April 28 and 23½ May 12th. During April and May she was perfectly well, except for being constipated, which condition grew worse. Toward the middle of June she became peevish and was apparently losing flesh. About June 25th, after the mother's milk was found by analysis to be deficient in fat, the child was placed on a diet of goat's milk, supplemented by a small portion of coddled egg, potato, etc. This change immediately relieved the constipation, which has given no further trouble. During July the baby was not very well, due perhaps to the cutting of several teeth. But from August on until October 1st she gradually gained in weight. October 4th the goat milk was replaced with cow's milk, but only for about six days, for she became quite sick, due perhaps to teething. Weight Nov. 10th, the end of the test, 25½ pounds. During this experience the milk has always digested easily. She has been a little slow about walking and teething as compared with other youngsters.

No. 6.— Mr.———

The child was six months old, weighed eight pounds, and in a very scrawny condition. Had not retained anything on its stomach to speak of for several days. The first feeding of goat's milk the child retained without the milk being adulterated, and it slept for ten hours, a thing the child had not done for weeks. For the 16 weeks the child was fed goat's milk, it averaged five-eighths of a pound per week gain. The child only gained three-eighths of a pound the first three weeks, which we considered very good for the condition she was in when we started. Since the child has been taken from goat's milk, it has had pure Holstein and is fat and perfectly healthy. She is now 21 months old and weighs 33½ pounds, is strong on her legs and has good teeth.

No. 7.— Dr.——— (Physician attending No. 6)

With reference to the use of goat's milk in the case of [No. 6], I would like to say that at the time this feeding was undertaken she showed marked signs of malnutrition and although various formulas had been tried she had gained scarcely anything over her birth weight when she was six months old. The goat's milk was used with my consent, not at my suggestion because I was not familiar with the possibility of obtaining a constant supply of this product. There are no doubt certain classes of cases in which goat's milk is a very suitable food for infants. This was one of those cases.

No. 8.— Mr.———

I am pleased to say that goat's milk has done wonders for my little girl. She was sick about five months and was nothing but skin and bones and could not keep anything on her stomach until we tried goat's milk. That seemed to agree with her and she began to pick up as soon as she began taking it. She is three years old in February, and is fatter than she ever was. I am thankful to you for the goat's milk.

No. 9.— Mrs.———

I began feeding my baby goat's milk from the Experiment Station about September 1, 1913, she being just then past three months old. I acted on the advice of Dr. .... of this city and consulted with him as to the preparation of the milk. In the first month the baby had the milk only as supplementary feeding and a very small amount at a time, from 1 to 2 ounces per feeding. There was no disturbance whatever of the digestive tracts from the mixed feedings. Stools were normal and free from curd. After expiration of first month breast milk decreased and amount of goat's milk was considerably increased, still however only used as supplementary feeding. By end of third month of feeding, that is by time infant was six months old, she was entirely weaned from breast. She had been all the time well, had no flatulence, and gained fairly regularly, but at no time rapidly, one-fourth pound per week being her average gain. For another month she remained on the goat's milk and from that time on she developed a very constipated condition which I attributed to deficiency in fats in the goat's milk. As the condition persisted, I decided to change to cow's milk (Holstein special at first, later a richer milk). She made the change without disturbance and is now at nine months in excellent condition and making normal gain. I have had a much easier time with her feeding than with that of my other bottle-fed babies who were fed cow's milk at about the same age.

No. 10.— Mr.———

It is a very great pleasure for me to tell you that the goat's milk which you furnished us for the past three weeks has worked wonders. Previous to when I talked with you, the baby had been under the care of two physicians with no apparent relief for several weeks. Since feeding goat's milk we have had no occasion to call a physician or give any medicine. The baby has gained in weight and his general condition is greatly improved.

No. 11.— (Further report on No. 10).

In summing up our report in the use of goat's milk which you furnished us, will say that when we first used the milk the baby was about five months old, when born it weighed 9½ lbs., gained while its mother nursed it to 12, and then when we put it on cow's milk it gradually went back to 9 lbs. At five months was very weak, threw up everything, worried continuously and had been under the care of two physicians (at different times) for two months and was completely at a standstill. We noted an improvement in his condition after the very first feeding of goat's milk and have never had a setback yet. He has never had any medicine since first using it and now weighs 16 lbs. at eight months.

No. 12.— (From attending physician)

I am sorry to report that the goat's milk did not agree much better with my little patient than cow's milk. He is a child 20 months old, who does not seem able to absorb anything. Taking 9 ounces a day of goat's milk was all he could care for without developing signs and symptoms of fat poisoning. Of cow's milk he could generally take 8 ounces a day, but sometimes even that amount seemed to poison markedly. I gave him for a time breast milk. Of that he could take 16 ounces a day, but was not poisoned even though fat appeared in his stools and the odor was rancid.

No. 13.— (From attending physician)

Two months ago ——— was put on goat's milk for part feeding,— being nursed also. When put on goat's milk weighed 15 lbs.; muscles firm, fairly nourished. Took the milk well, is now in good condition and weighs 17 lbs.

No. 14.— Mr. ———

I am having Mrs. ——— keep a record of the baby's condition since beginning to take goat's milk. I had expected to write this letter at home where I could get the data to send you. However, I will send you that later but wish to say now that he has gained 4 ounces in the past week and has been happier and more easily cared for than at any time since last fall. I believe the milk is helping him. He is now about 18 months old and we are feeding him some [proprietary food], eggs and crackers and a little bacon during the day, though the amount is very small. These we were feeding him before we obtained goat's milk, as the doctor thought that a child of his age should have some such food. We have substituted goat's milk for the cow's milk we were giving him. I think that is what is being of such benefit to him.

No. 15.— (First report)

I herewith give you report on condition of my little daughter in connection with the use of goat's milk, which is as follows:

Age — 4 months 19 days
Weight at birth 8½ lbs. (Feb. 15, 1914)
Weight........9¾ lbs. (April 26, 1914)
" ....9 lbs. (June 25, 1914)
Date started using goat's milk June 25th, weight 9 lbs.
July 3rd, " 9 lbs. 6 oz.

Net gain, 6 oz.

Upon advice of physician only used milk in proportion as shown below:
⅓ part sterilized milk
⅔ " " water
Starting July 3rd, will use as follows:
½ parts sterilized milk
½ " " water

As the proportion of milk is increased and that of the water decreased, expect that it will show a decided increase in weight.

No. 15.— (Second report)

In connection with the use of goat's milk as food for my young daughter, would advise that report for second week is as follows:

Weight on June 25th	9 lbs.
" " July 3rd	9 lbs. 6 oz.
" " " 10th	10 lbs. 6 oz.
Net gain	1 lb. 6 oz

The results thus far are very gratifying, I assure you, for we had used almost everything, and had despaired of saving her at all, until advised to procure the goat's milk. At present time we are using $\frac{2}{3}$ sterilized milk and $\frac{1}{3}$ sterilized water, and as she continues to improve will increase to full strength.

No. 15.— (Third report)

I herewith hand you statement in connection with use of goat milk for food of my young daughter, which is as follows:

Weight at start on	June 25th	9 lbs.
Weight	July 3rd	9 lbs. 6 oz.
"	July 10th	10 lbs. 6 oz.
"	July 17th	11 lbs. $2\frac{1}{4}$ oz.
Net gain		2 lbs. $2\frac{1}{4}$ oz.

Would also advise that we are using three-fourths milk now and only one-fourth sterilized water.

No. 16.— Mrs. ——— (First report, July 9, 1914)

We are very much pleased with the success of goat's milk with our baby during the month that he has been using it. He has gained ten ounces and is much happier and stronger. He is a year and a half old and only weighs thirteen pounds now. We have never been able to secure suitable food in spite of the most expert advice of noted baby specialists, so we feel that the goat's milk will prove to be a wonderful success in his case. Mr. ——— and I are very grateful to you for your kind help and interest in our behalf.

No. 16.— Mrs.——— (Second report, December 14, 1914)

I hope you will pardon my delay in sending you the results of our use of goat's milk for our baby. He was a year and a half old when we began using it in June. Up to that time nothing could be found to agree with him. He has been under the care of a famous baby specialist for weeks but with no results. He began to improve at once, gaining from four to ten ounces a week. In less than three months he had gained from a weight of twelve and a half pounds to over fifteen. At that time we found that he could handle cow's milk successfully. He has been making good progress since and has gained both in weight and strength. We feel that the use of goat's milk was of wonderful benefit to him, and we wish to thank you for your kindness in sending it.

No. 17.— Mr. ———

Last fall our baby was quite ill, and although we tried all kinds of modified milk and patented foods, we were unable to find any food which would nourish it. Finally thru your courtesy and that of Mr. ———, we tried goat's milk from the Experiment Station which was sent to us every day for several weeks. This the baby seemed to assimilate and digest perfectly, and it gave him a start from which he is still benefitting. I am taking this opportunity to express to you the appreciation of both Mrs. ——— and myself for your very great kindness in letting us have the goat's milk at that time.

No. 18.— (Report of attending physician)

In reference to the ——— child, I send you a few details. Should you wish I could send my notes, but I fear they would not be enlightening. It was 14 months old when I first saw it, weighing then about 17½ pounds, a loss of 3½ pounds during the past four months. It did well on a high-fat formula up to about nine months of age, and then suffered from constipation of a stubborn type. Later this turned to a diarrhoea, becoming a mild sub-acute catarrhal colitis, and presented all the usual symptoms of an intestinal indigestion, such as restlessness, pallor, languor, etc. Physical examination did not show anything grossly abnormal. The anterior fontanelle was too wide open. It had only four teeth and its muscles were very soft. Its intolerance for high fat was soon followed by a lack of tolerance of a very low fat of cow's milk. In other words, cow's milk in any form could not be given in sufficient ration in its formulae. I first gave its stomach a rest from all forms of milk, using [proprietary food]. I then started replacing gradually the [proprietary food] by the goat's milk you so generously provided. It progressed very well and was soon able to take more goat's milk in its formulae than it had ever taken cow's milk. It was also given flour ball and later some yolk of egg. Finally it was doing so well on a diet normal for its age that Mrs. ——— stopped writing to me. I do not remember seeing the baby after the first visit.

MILKING MACHINES.*

MAY BE OF GREAT SERVICE DURING THE PRESENT LABOR SHORTAGE IF USED ON FARMS WHERE 20 OR MORE COWS ARE KEPT.

SELECTING A MACHINE.

Use the same business judgment that you would in buying other machines. If the agents tell you the same stories that they have told to us, many of them should be accepted with a grain of salt. The longer a machine has been in successful operation, the surer you can be that it is a mechanical success. A record of three years of successful operation is none too long to make sure that a machine is worth buying; and the record should have been made independently of the manufacturer.

As a general rule, the simpler the construction of a machine, the more likely it is to be a success. This statement applies especially to teat cups. If of complicated construction, it is very difficult to keep them bacteriologically clean. Do not accept statements that machines which allow stable air to pass into the machine with the milk are failures because they are unsanitary. Our recent tests show that not more than 1 or 2 bacteria per c. c. are added to the milk in this way.

OPERATING A MACHINE.

Even if you have installed a machine which is as good as there is to be had, you may be sure it will not be a success on your farm unless you operate it properly. It is as necessary to use judgment and care in milking a cow by machine as by hand. The cow is not a machine and never can be made over into one. If you fail to make a success of a machine which others have used successfully for three or more years, the probabilities are that the trouble is with you. Instead of getting discouraged study the machine and try to discover what is wrong. We should be glad to pass on to you any information of value which we have gained from our ten years of experience with machine milking.

CAN YOU GET AS MUCH MILK BY MACHINE AS BY HAND?

Yes. Bulletin 353 answers this question. The records there given are by far the most extensive that have ever been gathered.

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* Reprint of Circular No. 54, May 10, 1917.

KEEPING THE MACHINE BACTERIOLOGICALLY CLEAN.

This is just as important as operating it properly. Very few farmers who are using machine milkers are keeping them bacteriologically clean. Where this is not done, the milk usually has a germ content of 50 to 10,000 per c. c. as it enters the teat cups and leaves them with a germ content of 200,000 to 5,000,000 per c. c. Such milk sours quickly and is not fit to be sold as market milk, or for butter or cheese making.

The pail can be kept clean in the same way that any milk pail is kept clean. *Steam or scald it and dry it out thoroly.* Rubber parts cannot be kept clean in this way and they should be kept in a disinfectant solution. Various germicides have been tried out for the purpose and several of them are useful. Very few have been tried out thoroly enough to justify recommending them. The one which we have found to be the most useful and which we know will give satisfaction *if directions are followed* is ordinary chloride of lime (bleaching powder) purchasable at any drug store. This is equally as good as or better than the patent preparations on the market frequently recommended by manufacturers of milking machines, for all of which you pay double prices.

PREPARATION OF DISINFECTANT SOLUTION.

Do not buy more than one or two 12 oz. cans of chloride of lime at any time and do not accept any in broken or rusted packages, or any that is moist. It should be a dry powder if it is fresh and of good strength. Prepare a stock solution by adding all of the powder in a can to a gallon of water in a pitcher or tall glass jar. This will give you a greenish colored liquid with a heavy, white sediment of lime.

Fill a second crock holding 20 to 30 gallons with water and add one pint of the stock solution to this *twice a week*. Double this quantity will do no harm. The solution in the big crock loses strength quickly on using and in a few days will become useless if the new chloride is not added. It is advisable to add enough salt to the crock to make a strong brine as this keeps the solution from freezing in cold weather, and brine is of itself a good solution in which to keep the teat cups (see Bulletin 353). The salt, however, is not necessary if attention is given to keeping up the strength of the chloride solution. This solution may be used indefinitely if its strength is maintained by adding fresh chloride of lime solution as directed.

PROCEDURE FOR CLEANING.

Immediately after each milking prepare three pails. Fill Pail 1, with clean cold water, Pail 2 with hot sal soda water, and Pail 3 with clean hot water. While the teat cups are still attached to the machine immerse them in these pails successively, at the same time sucking the water thru them. Then take the teat cups and stanchion hose and either suspend them or immerse them in the solution in the large crock. Care should be taken when putting the tubes into the solution to make sure that all air bubbles are out of the tubes and that they are completely immersed.

Neither rubber nor properly made metal parts are injured by the solution recommended. In case you have trouble from corrosion of the metal parts, it will show you that the manufacturer from whom you have purchased your machine has given little thought to making his machine a sanitary as well as a mechanical success. Our machines handled as above outlined have been found by many tests to be as near sterile as it is practicable to make them. Milk drawn thru machines cared for in this way is cleaner and freer from bacteria than hand drawn milk.

Once a week the rubber teat cups should be taken apart completely and each part thoroly cleaned. *No disinfecting solution will take the place of cleaning.*

Just before beginning to milk, suck a pail of clean water, either hot or cold, thru all of the teat cups. Otherwise traces of the disinfectant may be carried over into the milk. Even if this does happen and traces are carried over, harmless compounds are formed like some already present in the milk. The action is such that it would be necessary to add large quantities of the disinfectant if anyone should attempt to use it fraudulently as a preservative in milk. If this is done the milk has such an unpleasant odor and taste that it is unsalable. Chloride of lime is also a very valuable disinfectant for use in caring for unfiltered city water supplies and large quantities of it are used in this way.

DO MILKING MACHINES SPREAD OR CAUSE GARGET?

It is frequently claimed that they do but there is no satisfactory evidence upon which to base such a claim. Thus far very few records have been secured upon which to base an intelligent opinion. We have had no more trouble with garget in the Station herd in the case of machine-milked cows than in the case of hand-milked cows. Moreover, such records as we have been able to gather in the course of milk control work where we examined the milk from 36–40 farms (eight of which have used or are using machines of four different makes) does not indicate that garget is spread any worse in the machine-milked than in the hand-milked herds. In the course of two years four herds have been badly affected with garget. Two of these were hand milked, two machine milked. Machine milking was discontinued on one farm partly on account of the garget. On the other farm machine milking was continued and the garget infection cleared up fully as quickly as it did on the farms where hand milking was practiced. Further information secured under carefully controlled conditions must be obtained before it will be clear whether or not this claim that garget is spread by machine milking is true. There is no evident reason why it should be so.

FINAL WARNING.

The labor shortage has caused and will cause machines to be put upon the market which are intended to sell rather than to give satisfaction. Deal only with responsible firms whose business reputation is worth more to them than the few thousand dollars which can be gained by selling a few milking machines.

N. B.—The above is a preliminary report of experiments still in progress. A more complete report will be published in bulletin form when the work is completed.

REPORT

OF THE

Department of Bacteriology.

R. S. Breed, *Bacteriologist.*
H. J. Conn, *Associate Bacteriologist.*
G. L. A. Ruehle, *Assistant Bacteriologist.*
J. D. Brew, *Assistant Bacteriologist.*
John Bright, *Assistant Bacteriologist.*

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I. Soil flora studies, Parts I to V.
II. What is meant by quality in milk?
III. The number of bacteria in milk.
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REPORT OF THE DEPARTMENT OF BACTERIOLOGY.

SOIL FLORA STUDIES.

H. JOEL CONN.

GENERAL INTRODUCTION.

During the past twenty years or more, considerable attention has been given to the relation of bacteria to nitrogen-transformations in soil and in manure. This particular phase of soil microbiology has been so extensively studied because of the manifest importance of nitrogen in the nutrition of plants. There still remain, however, many other problems connected with the micro-organisms in the soil that are of fundamental importance and which must be studied before a true appreciation of the significance of soil bacteria to agriculture is possible.

There are, for example, many unanswered questions relating to the distribution, function, and associative action of the micro-organisms known to be abundant in soil. These questions require a method of attack almost the opposite from that used in studying the nitrogen-transformations. The latter type of investigation starts with a known function and is directed toward the unknown organisms (or at least unknown at the outset) to which this function belongs; the other type starts with known organisms and is directed toward their unknown functions. The study of nitrogen-transformations has been centered around a few special physiological activities; while the other type of study is concerned with the general characteristics of the entire soil flora. Therefore, investigations of the latter sort may be distinguished from those of the former by calling them soil flora studies.

In making a study of the soil flora, the first step necessary is classification. Investigations relating to the distribution or function of the micro-organisms cannot proceed far without a thoro knowledge of the kinds of organisms in question. It is not yet possible to make a complete classification of the microscopic flora of soil; but it is possible to make a preliminary grouping of the organisms. Nothing more than this is attempted in the present work; but it is hoped that this preliminary grouping may be used by later investigators and developed by them into a more extensive classification.

The results now ready for publication are to be written up in a series of five papers. The present bulletin, containing the first two of these papers, is really an introduction to the three that are to follow. The three later papers are to be published in separate bulletins, and each one is to take up one of the three most important groups of soil bacteria: Actinomycetes, spore-forming bacteria, and non-spore-forming bacteria. An outline of these papers follows the "Acknowledgments."

ACKNOWLEDGMENTS.

This work was begun at the suggestion of Dr. H. A. Harding (formerly Bacteriologist at this Station), whose criticisms and advice proved most valuable during the two years while the work was proceeding under his direction. During the last three years the helpful suggestions of Dr. R. S. Breed have also proved of great value. Equal acknowledgments are due to the writer's father, Dr. H. W. Conn, for the inspiration of conversation and correspondence with him in regard to the work. Lastly, credit is due to Dr. W. W. Ford, who has given much valuable assistance in determining the species of spore-forming bacteria encountered in this work.

OUTLINE OF THE SOIL FLORA STUDIES.

TECHNICAL BULLETIN NO. 57.

I. The general characteristics of the microscopic flora of soil.
  - Previous investigations.
  - Soils investigated.
  - Kinds of micro-organisms in soil.
    - Limitations of the study.
    - Peculiarities of the soil flora.
    - Groups of micro-organisms found.
    - Importance of these different groups.
  - Conclusions.

II. Methods best adapted to the study of the soil flora.
  - Introduction.
  - Sampling soil.
  - Plating soil for quantitative purposes.
    - General technic.
    - Comparison between soil-extract gelatin and tap-water gelatin.
    - Comparison between different brands of gelatin.
    - Comparison between plain asparaginate agar and asparaginate agar with glycerin.
    - Comparison between gelatin and agar.
  - Plating soil for qualitative purposes.
    - Advantages of gelatin.
    - Advantages of agar.
    - Technic at present recommended.
  - The study of pure cultures.
    - The classification card of the Society of American Bacteriologists.
    - The group number.
    - Value of the card in studying soil bacteria.
    - Diagnostic value of the tests given on the card.
    - Reasons for the inconsistent results.
  - Conclusions.

TECHNICAL BULLETIN No. 58.

- III. Spore-forming bacteria in soil.
  - Summary.
  - Introduction.
  - Historical.
  - Classification.
    - The three most common species.
      - *B. megatherium* DeBary.
      - *B. mycoides* Flügge.
      - *B. cereus* Frankland.
    - Less common species.
    - Gelatin colonies of the spore-forming bacteria.
  - Significance of the spore-forming bacteria in soil.

TECHNICAL BULLETIN No. 59.

- IV. Non-spore-forming bacteria in soil.
  - Summary.
  - Introduction.
  - Historical.
  - Classification.
    - The two principal groups.
    - Rapid liquefiers.
      - *Ps. fluorescens* (Flügge) Migula.
      - Orange liquefying type.
      - Other types.
    - "Slow-growers."
      - General characteristics.
      - Rod-shaped forms.
      - Cocci.
    - Recognition of colonies on plates.
  - Distribution.
    - Abundance in soil.
    - Fluctuations in numbers.
  - Significance of the non-spore-forming bacteria in soil.

TECHNICAL BULLETIN No. 60.

- V. Actinomycetes in soil.
  - Summary.
  - Introduction.
  - General description of Actinomycetes.
  - Historical.
    - The question of nomenclature.
    - Early attempts at classification.
    - Krainsky's work.
    - Waksman's work.
  - Classification.
    - Methods used in the present work.
      - Morphological criteria.
      - Growth on glycerin media.
      - Growth on other media.
      - Weakness of present methods.
    - The most abundant types in soil.
      - The pink-spored type.
      - The dark-brown type.
      - The potato scab type.

V. Actinomycetes in soil (*continued*).
  Classification (*continued*).
    The numerous less common types.
    Recognition of colonies on plates.
  Distribution.
    Occurrence in nature generally.
    Occurence in soil.
  Significance of the Actinomycetes in soil.
Discussion of the whole series of soil flora studies

SOIL FLORA STUDIES.*

I. THE GENERAL CHARACTERISTICS OF THE MICROSCOPIC FLORA OF SOIL.

II. METHODS BEST ADAPTED TO THE STUDY OF THE SOIL FLORA.

H. JOEL CONN.

SUMMARY.

1. A study has been made of the microscopic flora of various New York State soils. The work has been in progress for about eight years.
2. Determinations of the total number of bacteria have been made by the plate method. Little stress has been laid on the quantitative results, however, as the method is considered to be inaccurate.
3. The chief use made of the plate method has been to learn the relative numbers of different kinds of organisms in the soil, so far as it is possible to distinguish them from each other by the appearance of their colonies on the plates.
4. About one thousand pure cultures of these different organisms have been isolated and studied by various methods to learn their characteristics.
5. Non-spore-forming bacteria (mostly immotile rods) have been found to be the most abundant of all soil micro-organisms. Next to them in abundance are the various types of Actinomyces.
6. Certain spore-forming bacteria have also been found, but not in great numbers; and apparently they are not of great importance in normal soil.
7. Soil bacteria are quite different from those found in milk, cheese, or water. Evidently soil has its own characteristic bacterial flora.

I. GENERAL CHARACTERISTICS OF THE SOIL FLORA.

PREVIOUS INVESTIGATIONS.

Soil flora studies in the past — and the same fact is true concerning flora studies of milk or water — have developed in two different directions, the first extensive, the second intensive. Extensive studies have resulted in learning a little about many kinds of soil organisms; intensive studies in learning much about a few, small, selected groups of organisms. Both lines of work must be harmonized in order to obtain a comprehensive knowledge of the soil flora.

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* Reprint of Technical Bulletin No. 57, January, 1917

An early contribution to the extensive flora studies of soil was made by Houston.[1] This line of work was further developed by Chester in 1903,[1A] when he outlined the principles involved in such a study and the methods necessary. In an earlier publication[2] he had given rather full descriptions of miscellaneous bacteria isolated from soil; but it was in 1903 that he first emphasized the importance of determining the predominating soil organisms and selecting them for special study. In 1904,[3] he carried this line of investigation to an extreme in which he has not been followed by other investigators. At this time he proposed a formula (coefficient of "zymotic efficiency") for expressing the results of qualitative bacterial analysis of soil. No subsequent worker has expressed his results in the terms proposed by Chester; but this does not alter the fact that important principles underlie Chester's work. He was handicapped by the lack of intensive work on the various groups of soil organisms (a handicap which he realized and did his best to overcome by intensive studies of his own), and also by the crudity of bacteriological methods at the time when his work was done. In spite of these handicaps, Chester's work still stands as the most complete extensive study yet made of the soil flora; and it is unfortunate that he was forced to drop this line of work before it had progressed far enough to have practical results.

At about the same time that Chester was making these studies, Hiltner and Störmer[4] published a piece of work which did not go as far as Chester's work in classifying and describing the different kinds of bacteria, yet which has proved as valuable to later investigators as Chester's contributions. Instead of using the meager data at their command as a basis for establishing species, they merely pointed out three large groups of soil micro-organisms that can be readily distinguished on gelatin plates and showed that the relative abundance of these three groups is nearly constant in normal soil. From their data it appeared that any external influence which disturbed the equilibrium of the soil flora would be indicated by a change in the relative abundance of these three groups. This conception of the soil micro-organisms as being normally in a state of equilibrium has proved of considerable value in interpreting soil phenomena.

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[1] Houston, A. C. Chemical and bacteriological examination of soils. Local Gov't Board, Rept. 27:251–296. 1898. The results of this investigation are considered primarily in their relation to pathogenic organisms and the general conclusions are of less interest than the details of the work. The paper will therefore be more fully discussed in Tech. Buls. 58–60.

[1A] Chester, F. D. Study of the predominating bacteria in a soil sample. Del. Agr. Exp. Sta., Rept. 14:52-66. 1903.

[2] Chester, F. D. Description of certain species of bacteria isolated from cultivated soil. Del. Agr. Exp. Sta., Rept. 11:52–75. 1900.

[3] Chester, F. D. Bacteriological analysis of soils. Del. Agr. Exp. Sta., Bul. 65. 1904.

[4] Hiltner, L., and Störmer, K. Studien über die Bakterienflora des Ackerbodens, mit besonderer Berücksichtigung ihres Verhaltens nach einer Behandlung mit Schwefelkohlenstoff und nach Brache. *Kaiserl. Gesundheitsamte, Biol. Abt. Land- u. Forstw.*, 3:445–545. 1903.

Meanwhile intensive work on special groups of soil bacteria has been accumulating more rapidly than extensive work on the general flora. Part of this work has been done on the group of spore-forming bacteria, and part of it on Actinomyces. Chester, himself, contributed to the study of the spore-formers.[5] Also a series of articles on this group has been appearing from time to time representing the work of Meyer [6] and his students at Marburg (Gottheil,[7] Neide [8] and Holzmüller [9]). Certain disagreements between Chester's work and the Marburg work have caused some confusion for others who have wished to study the group; so the recent work of Ford and his associates,[10] which harmonizes previous investigations, is an especially welcome contribution.

The Actinomycetes have been less carefully studied. Nearly all the work done on this group has been on the pathogenic types, and the early systematic work (e. g. that of Sauvageau and Radais,[11] Gasperini,[12] Lachner-Sandoval [13] and Sanfelice [14]) left much to be desired. Recently, however, an important article by Krainsky [15] has opened the field for further investigations of this group. His methods are already beginning to be used by other investigators.

A more complete discussion of the literature on spore-formers and Actinomycetes will follow in the two later papers of the present series which are to take up these two groups of organisms.

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[5] Chester, F. D. Observations on an important group of soil bacteria: Organisms related to *Bacillus subtilis*. Del. Exp. Sta. Rept. **15**:42–96. 1904. A review of the *Bacillus subtilis* group of bacteria. *Centbl. Bakt.*, II Abt., **13**:737–752. 1904.

[6] Meyer, A. Practicum der botanischen Bakterienkunde. Jena. 1903.

[7] Gottheil, O. Botanische Beschreibung einiger Bodenbakterien. *Centbl. Bakt.*, II Abt., **7**:430, 449, 481, 529, 582, 627, 680, 717. 1901.

[8] Neide, E. Botanische Beschreibung einige sporenbildenden Bakterien. *Centbl. Bakt.*, II Abt., **12**:1, 161, 337, 539. 1904.

[9] Holzmüller, K. Die Gruppe des Bacillus mycoides Flügge. *Centbl. Bakt.*, II Abt., **23**:304–354. 1909.

[10] Ford, W. W., et al. Studies on aerobic spore-bearing non-pathogenic bacteria. *Jour. Bact.*, **1**:273–320, 493–534. 1916.

[11] Sauvageau and Radais. Sur les genres Cladothrix, Streptothrix, Actinomyces. *Ann. Inst. Past.*, **6**:242–273. 1892.

[12] Gasperini, G. Versuche über das Genus Actinomyces. Abstract in *Centbl. Bakt.*, I Abt., **15**:684–686. 1894.

[13] Lachner-Sandoval. Ueber Strahlenpilze. Inaug. Diss. Strassburg. 1898.

[14] Sanfelice, F. Ueber die pathogene Wirkung einige Streptothrix- (Actinomyces-) Arten. *Centbl. Bakt.*, I Abt., Orig., **36**:355–367. 1904.

[15] Krainsky, A. Die Aktinomyceten und ihre Bedeutung in der Natur. *Centbl. Bakt.*, II Abt., **41**:649–688. 1914.

Thanks to the intensive work which has been done on these two groups, extensive work on the general soil flora can be undertaken today with a much greater chance of success than in earlier years. Flora studies have already proved of great value in dairy work; as shown by the investigations of H. W. Conn, Esten and Stocking,[16] Harding and Prucha,[17] and Hastings, Evans and Hart,[18] which have resulted in showing the significance of the lactic acid types in milk and cheese (in milk the common lactic acid type, and in cheese the common type and also the Bulgarian type). Knowledge of equal importance in regard to soil may easily result from a better acquaintance with the soil flora.

Realizing this, several years ago the writer criticised the prevailing methods of soil bacteriological investigations, pointing out the importance of making a flora study, and concluded with the words: "In the future, if we wish to make any great advance in our knowledge, we must learn first to distinguish as rapidly as possible the different bacteria present in any given soil and then must study the significance of the forms we have learned to recognize." [19] Following up the line of work indicated in this statement, a preliminary grouping of the bacteria in a certain soil was published a few years ago.[20] This study included all the forms obtained upon gelatin plates incubated in the presence of air. About thirty-five types of bacteria were mentioned that could be distinguished by means of certain cultural and physiological characteristics. It was expected that some of these types could later be broken up into two or more species, while others would prove to be separated by characteristics not sufficiently constant to furnish actual distinctions. This study has been continued for five years since the work already published was brought to an end; and now considerable information is at hand to show which of these types are distinct, which must be grouped together, and which require further subdivision.

During the course of this study, several minor points have been investigated and discussions of them have been published from time to time. The surprisingly high plate counts of frozen soil, for example, were noticed early in the work, and as data accumulated on this subject, they were published.[21] Gradually, as the work progressed, evidence was obtained of the comparative value of different culture media for soil bacteriological work.[22] These minor publications all had to do with quantitative work alone (altho the main line of investigation was primarily qualitative), because results could be obtained with greater ease in quantitative work. It is only recently that the qualitative side of the work has been advanced sufficiently for publication. Two short articles have been published within the last year, one dealing with the spore-formers of soil [23] and the other with Actinomycetes.[24] The work published in all these papers formed an integral part of the general soil flora study; so it is all summarized in the present series of publications.

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[16] Conn, H. W., Esten, W. M., and Stocking, W. A. A classification of dairy bacteria. Storrs (Conn.) Agr. Exp. Sta., Rept. 1906 : 91–203.

[17] Harding, H. A., and Prucha, M. J. The bacterial flora of cheddar cheese. N. Y. Agr. Exp. Sta., Tech. Bul. 8. 1908.

[18] Hastings, E. G., Evans, Alice C., and Hart, E. B. Studies on the factors concerned in the ripening of cheddar cheese. Wis. Agr. Exp. Sta., Res. Bul. 25. 1912.

Bacteria concerned in the production of the characteristic flavor in cheese of the cheddar type. *J. Agr. Res.,* **2**:167–192. 1914.

[19] Conn, H. J. Future methods of soil bacteriological investigations. *Centbl. Bakt.,* II Abt., **35**:454–457. 1909.

[20] Conn, H. J. A classification of the bacteria in two soil plats of unequal productivity. Cornell Agr. Exp. Sta., Bul. 338 : 65–113. 1913.

SOILS INVESTIGATED.

In the course of this investigation, about five hundred samples have been taken from as great a variety of soils as could be obtained in the neighborhood of the Experiment Station. This included muck, clay loams, loams, sandy loams and fine sand. With the exception of the muck, these soils were all of glacial origin or of post-glacial alluvial derivation. They are, for the large part, delta deposits in one of the glacial lakes of central New York. Described according to the system of the Bureau of Soils of the U. S. Department of Agriculture,[25] these soils are as follows:

- Glacial lake deposit:
    - Dunkirk loam,
    - Dunkirk fine sandy loam,
    - Dunkirk sandy loam,
    - Dunkirk gravelly loam,
    - Dunkirk fine sand;
- Glacial till from the New York drumlin area:
    - Ontario loam,
    - Ontario fine sandy loam;
- Glacial till from Devonian shales and sandstones:
    - Volusia silt loam;
- Alluvial:
    - Genesee silty clay loam,
    - Genesee loam;
- Limestone residual, mixed somewhat with glacial materials:
    - Honeoye stony loam.

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[21] Conn, H. J. Bacteria of frozen soil. *Centbl. Bakt.*, II Abt., 28:422–434, 1910; 32:70–97, 1912; 42:310–519, 1914. N. Y. Agr. Exp. Sta., Tech. Bul. 33, 1914.

[22] Conn, H. J. Culture media for use in the plate method of counting soil bacteria. N. Y. Agr. Exp. Sta., Tech. Bul. 38. 1914.

[23] Conn, H. J. Are spore-forming bacteria of any significance in soil under normal conditions? N. Y. Agr. Exp. Sta., Tech. Bul. 51. 1916. *Jour. Bact.*, 1:187–195. 1916.

[24] Conn, H. J. A possible function of Actinomycetes in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 52. 1916. *Jour. Bact.*, 1:197–207. 1916.

[25] U. S. Dept. Agr. Bureau of Soils Bul. 96, pp. 1–791. 1913. See also Survey of Ontario County, New York, published by this Bureau, 1912; pp. 1–55.

These soils were mostly agricultural soils, but some of the muck samples were taken from woodland. The samples were taken from soils in various states of cultivation, including sod of various ages and cultivated soil under various crops. Many of the samples were so taken as to furnish a comparison between sod and cultivated portions of the same soil.

KINDS OF MICRO-ORGANISMS IN SOIL.

Limitations of the study.— At present it would be a hopeless task to make a complete study, or even a complete classification, of the various types of micro-organisms occurring in soil. Because of the necessity of setting some limit to the work, only those forms have been studied that grow in the presence of air and occur in appreciable numbers on plates inoculated with soil infusion diluted 100,000 times. This excludes a number of the soil bacteria that have been described by others.

Foremost among the soil bacteria that are considered important, yet which are not mentioned in the present work, are those concerned with the transformations of nitrogen. The nitrifiers, for instance (which convert ammonium salts into nitrites and nitrates), do not grow on the ordinary organic laboratory media, and for that matter have never been found in soil in sufficient numbers to occur on plates dilute enough to show the more abundant organisms. The denitrifiers (that reduce nitrate to elementary nitrogen) and the nitrogen-fixing bacteria (that are able to assimilate free atmospheric nitrogen) likewise occur in too small numbers to be found by the methods employed in this work.

The bacteria that produce nodules upon the roots of legumes and enable these plants to utilize atmospheric nitrogen are excluded from this study, altho they can be obtained upon plates made directly from soil in the dilutions employed in the present work (as shown by Lipman and Fowler[26]). They have not been encountered in the course of this work, however, probably because no special search has been made for them and because the media used have not been particularly adapted to them.

As all of the plates were incubated in the presence of air, strict anaerobes, if such occur in the soil, have been overlooked. The importance of strict anaerobes in soil, however, is somewhat questionable. The peculiar structure of soil seems to make it pre-eminently a medium for the growth of aerobic bacteria. It has often been claimed in the past that water-logged soil furnishes favorable conditions for anaerobes, particularly in the deeper layers. This is not at all improbable; but normal soil seems to furnish more favorable conditions for aerobic growth than any other natural medium inhabited by bacteria.

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[26] Lipman, C. B., and Fowler, L. W. Isolation of *Bacillus radicicola* from soil. *Sci.,* N. S., 41:256–259. 1915.

Evidence has been obtained which supports this conclusion. Plates grown in the absence of oxygen always show a very scanty flora, a few thousand per gram; and none of the cultures isolated from the colonies on these anaerobic plates have proved to be *strict* anaerobes. Not enough evidence of this nature has been obtained to justify a positive statement; but the few results at hand are at least suggestive. They are borne out by the fact that the flora on ordinary aerobic plates consists almost wholly of strict aerobes, although the growth of facultative anaerobes should not be prevented by the presence of oxygen. The only facultative anaerobes encountered on aerobic plates are the spore-forming bacteria, and apparently they are not active in normal soil. If anaerobic growth were common in soil, it hardly seems as if there would be such a preponderance of *strict* aerobes on the plates. There is no reason, in view of the data obtained, for believing strict anaerobes to be of any real significance in normal soil.

Two other classes of organisms that may be important in soil, which have been excluded from the present work, are higher fungi and protozoa. The importance of protozoa in soil is not yet established, but a study of them would have been made except for the necessity of drawing some limits to the field investigated. Higher fungi have always been known to live in the soil, and their spores are numerous there; but it has never been shown whether mold hyphae are abundant enough in soil for their activities to compare with those of bacteria. The writer[27] has already called attention to this fact, and has stated that the microscope does not show the presence of mold hyphae in ordinary soil, altho it reveals the presence of bacteria in large numbers. Higher fungi certainly live in soil; but there is reason to doubt whether they are abundant enough to be of significance in comparison with bacteria.

A further limitation of the work has resulted from the fact that a complete classification of a flora as complex as that of soil — or even that part of it which develops on aerobic gelatin plates — offers difficulties that are almost insurmountable at the present day. Bacteriological methods are too crude as yet to allow the establishment of species, with any degree of certainty, even among pathogenic organisms and the bacteria found in milk and water. Among the bacteria of soil, which have been much less thoroly studied, there is still less certainty in regard to species. It is, therefore, much too soon to expect an exhaustive classification of the soil flora.

The most that can be looked for at present is a preliminary classification. This preliminary classification should include descriptions of known species that can be recognized without question;

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[27] Conn, H. J. Relative importance of fungi and bacteria in soil. *Sci.*, N. S., 44:857-858. 1916.

but to describe new species seems unwise. The literature relating to soil bacteriology is full of names of species in regard to which too little information is given to make recognition possible. It seems unwise to increase this confusion by adding more new names. The best plan seems to be to classify the soil micro-organisms into groups or types as small as present methods allow, each of which may be the subject of future investigation. Some of these types are undoubtedly species; others are larger groups, and must be further subdivided in the future.

Peculiarities of the soil flora.— One of the most striking characteristics of the soil flora that has come to light in the course of this work is its uniformity. Quantitatively this uniformity is particularly striking, as the number of colonies developing on gelatin or agar plates incubated at 18° C. for seven days is ordinarily between five and fifty million per gram of soil. Qualitatively there is also considerable uniformity, as pointed out some time ago.[28] It was stated then that the predominating types of bacteria in every kind of soil studied had proved to be the same, so far as they could be determined by the methods used. There is no reason yet for modifying that statement, altho since that paper was written new methods of study have allowed further classification of the Actinomycetes, and some of the types picked up in some particular soils have not yet been encountered elsewhere. It is perfectly possible that the same fact may prove to be true of other groups of soil organisms as better methods are adopted for their classification; but the statement can safely be made that the same general types of bacteria occur in all of the soils studied. The greatest difference yet observed has been in the relative abundance of the different groups present. This fact gives still more weight to Hiltner and Störmer's conception of an equilibrium among soil micro-organisms.

Another fact which has been noticed in making a study of the soil flora is that the organisms encountered are strikingly different from those found in other natural media inhabited by bacteria. The short rods and cocci that produce acid from sugars, which are so abundant in milk, are entirely lacking in soil. The large spore-forming bacteria with peritrichic flagella, which seem to be characteristic of water, occur in soil, it is true, but comprise only a small and apparently unimportant part of the total flora. The bacteria found in soil in the course of this investigation have proved to be mainly of two classes: (1) Actinomycetes, a group occurring in milk and water so rarely that its occasional presence always seems accidental; (2) small immotile rods without spores, that do not acidify sugars and grow slowly in all of the ordinary bacteriological media (liquefying gelatin slowly if at all), a group that does not occur in milk and has never been mentioned as occurring in water. The former group generally comprises 12–50 per ct. of the soil flora, the latter 40–75 per ct.

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[28] Conn, H. J. The distribution of bacteria in various soil types. *Jr. Amer. Soc. Agron.*, 5:218–221. 1913.

Groups of micro-organisms found.— Besides these two predominating groups, certain other bacteria have been found quite regularly but in smaller numbers. They are: Spores of the peritrichic spore-formers (often called the *B. subtilis* group); rapidly liquefying rods with polar flagella (principally *Ps. fluorescens*); and slow-growing cocci that liquefy gelatin slowly or not at all and do not ordinarily acidify sugars. A grouping of the soil bacteria which includes all these forms may be drawn up as follows:

Spore-producers;	"Rapid liquefiers."
Non-spore-producers;	
Liquefaction rapid;	
Liquefaction slow or none	"Slow growers."
Rods:	
Yellow chromogenic;	
Non-chromogenic;	
Cocci.	
Actinomycetes - - - -	Actinomycetes.

The three large groups, "rapid liquefiers," "slow growers" and Actinomycetes, given in the right-hand column of the above table, have been mentioned in several previous publications.[29] Except for the Actinomycetes, they are purely artificial groups, used because of the convenience with which they can be recognized from gelatin plates. Hiltner and Störmer [30] used a similar classification of their gelatin colonies. They state that gelatin plates from soil normally show 5 per ct. liquefiers, 70 per ct. non-liquefiers, and 20 per ct. Streptothrix. Their 5 per ct. liquefiers include the *B. subtilis* and *Ps. fluorescens* groups — the "rapid liquefiers." It is true that usually more than 5 per ct. of the colonies have been found to be rapid liquefiers in the present work; but Hiltner and Störmer do not state the percentage of gelatin used in their work, and a sufficient concentration of gelatin might easily have lowered the numbers to 5 per ct. in the present work. Hiltner and Störmer's "non-liquefiers" are undoubtedly the same as the writer's "slow growers," in other words the organisms that produce punctiform colonies on gelatin. The term "non-liquefiers," however, is misleading because many of them do liquefy gelatin in stab culture if given time enough and even produce liquefied colonies on the plates if the concentration of the gelatin is not too great. The average proportion of the total flora comprised by the organisms that produce punctiform colonies is not far from the 70 per ct. which Hiltner and Störmer find to be non-liquefiers. Their third group, " Streptothrix," is without question the same as the Actinomycetes. Streptothrix is, however, a poor term to use, as it was applied to another genus before it was used for this group of organisms. In cultivated soils, such as Hiltner and Störmer were studying, the Actinomycetes have been found to average almost exactly 20 per ct. as shown in a recent publication.[31]

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[29] See footnotes 20 and 21.

[30] Loc. cit., footnote 4.

The soil flora may also be characterized by the kinds of organisms that are lacking. No non-liquefiers producing acid from sugar have been observed. Organisms producing gas from sugar may possibly be present in small numbers,[32] but are found only rarely by the methods used in this work. The only organisms observed that are not strictly aerobic are some of the spore-formers, and they do not seem to be active in normal soil.

Importance of these different groups.— Unfortunately, little is known of the importance of these groups of soil organisms. Conclusions drawn from the relative abundance of the colonies on the plates are likely to be misleading. In the past, for example, when methods of plating were such as to prevent large numbers of the non-spore-formers and Actinomycetes from developing on the plates, the relative abundance of the spore-formers seemed to be quite high, and it was concluded that they must be important in the soil. The present work, however, as pointed out in a previous publication,[33] shows that they are actually present in comparatively small numbers and, even at that, occur ordinarily only in the form of spores. In just the same way future improvements in methods may show that the organisms apparently most abundant are not actually the most important micro-organisms in soil.

Nevertheless, there is good reason to believe that non-motile non-spore-forming bacteria, which show up in such abundance on gelatin plates, are actually, as well as apparently, the most numerous soil micro-organisms. It is also safe to conclude that the Actinomycetes are one of the more abundant groups of organisms in soil. If abundance bears any relation to importance, these are probably the two most important groups of soil micro-organisms; and indeed (as mentioned in a previous publication [34] and as will be further discussed in a later paper of this series), the relative numbers of these two groups of organisms show such a close relation to certain soil conditions that there can be little question as to their activity and importance in soil.

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[31] Loc. cit., footnote 24.

[32] They have been isolated from soil by others, as for example Chester's *Bact. fermentationis* (Del. Agr. Exp. Sta., Rept. 11, p. 53); but neither Chester nor anyone else has stated them to be among the predominating types.

[33] Loc. cit., footnote 23.

[34] Loc. cit., footnote 24.

CONCLUSIONS.

There is a characteristic microscopic flora of soil, which is different from that of any other natural medium. The following groups of micro-organisms develop on aerobic gelatin plates inoculated with soil:

I. From 5 to 10 per ct. spore-formers (the *B. subtilis* group). Nearly all the colonies of these bacteria, however, seem to come from spores instead of from active organisms.

II. Under 10 per ct. rapidly liquefying, non-spore-forming, short rods with polar flagella (principally *Ps. fluorescens*).

III. From 40 to 75 per ct. slowly liquefying or non-liquefying, non-spore-forming, short rods.

IV. A few micrococci. In cultural characteristics these are almost identical with the last mentioned group.

V. From 12 to 50 per ct. Actinomycetes.

Of these five groups, the most important ones seem to be Nos. III and V. This conclusion is based upon the following facts: These two groups are always present in large numbers; of the other three groups, the only one always present in large enough numbers to be detected on the plates is No. I, the group of spore-formers, which apparently exists in normal soil only in the form of spores.

II. METHODS BEST ADAPTED TO THE STUDY OF THE SOIL FLORA.

INTRODUCTION.

The technic for soil bacteriology has never been standardized. In dairy bacteriology certain official methods are recognized, which are quite rigid even in small details; but there have been no serious attempts to propose official methods for soil study. The reason for this difference is partly because soil methods are still in their experimental stage, and partly because no extensive routine examinations of soil are ever made. If such examinations were made, they would create a demand for the general use of methods that could be counted on for comparable results. It is really fortunate that no official methods are needed, as each investigator in soil bacteriology is free to develop his own technic; but as a result the investigator must always publish a detailed description of the methods he uses. The present paper is a discussion of the methods used in these Soil Flora Studies, the reasons for using them, and the lines along which these methods are likely to develop in the future.

SAMPLING SOIL.

The exact method of taking the soil samples has not been considered a matter of great importance. It was ordinarily unnecessary to secure a representative sample, as the object of the work was to find as many kinds of bacteria as possible rather than to make a quantitative comparison between the different soils. Nevertheless care was generally taken to make borings at different spots in the plat sampled and to mix thoroly all the soil obtained from these borings. Samples of field soil were generally taken with an auger to the depth of six inches. Pots were sampled with a cheese-sampler long enough to extend to the bottom of the pot; and a little soil was carefully selected from along the entire length of the core thus extracted. It was considered unwise to make more than one boring in a pot at each sampling, because of the amount of aeration thus produced.

Sterilization of the sampling tools was also considered unnecessary. They were always well cleaned, but not sterilized. With the great dilutions used, there is no reason for supposing that contaminations due to unsterilized sampling tools would ever cause the occurrence of more than one or two colonies per plate; and no type of organism has been considered in this work unless it has been of frequent occurrence and has appeared on the plates in more than isolated colonies. It was soon found, moreover, that the soil flora was so constant that any gross contamination could be easily detected by means of the presence of unusual types of colonies on the plates.

PLATING SOIL FOR QUANTITATIVE PURPOSES.

General technic.— The only satisfactory method yet devised for determining the numbers of bacteria in soil is by means of poured plates. It has long been recognized that this method does not give a complete count. There are bacteria in soil that do not grow in our ordinary culture media, and as this method gives a count of those organisms alone that are able to produce macroscopic colonies in agar or gelatin, those that do not grow are overlooked. Moreover, if the bacteria in soil occur in clumps that do not break up while the material is being prepared for plating, each clump produces but one colony, no matter how many individuals it may contain. Still further errors are introduced into counts made this way by the fact that differences in technic cause variations in the number of bacteria able to produce colonies.

This last source of error was taken up at some length in Technical Bulletin No. 38.[35] At that time the importance of a long incubation at a low temperature was emphasized. Gelatin plates, it was stated, should be incubated at temperatures as low as 18° C. for at least seven days, while agar plates should be held at least as long if not longer. Considerably lower counts were obtained with shorter periods of incubation.

Since that paper was published, some further work has been done to see what sort of incubation is best for agar. As agar can be kept at a higher temperature than gelatin, it seemed possible that more satisfactory results might be obtained in a warmer incubator. The results of this test are given in Table I. The two temperatures used

TABLE I.—TESTS TO DETERMINE THE BEST TEMPERATURE FOR INCUBATION OF AGAR
(Soil used was Dunkirk Silty Clay loam, kept in the laboratory in pots.)

Test No.	Date.	Medium used.	Colonies developing per gram of dry soil with			
	1915.	Asparaginate agar with:	Incubation at 18° C.		Incubation at 25° C.	
			7-day count.	14-day count.	7-day count.	14-day count.
1....	Nov. 15	1% glycerin....	26,000,000	***30,000,000**	23,500,000	23,500,000
2....	Nov. 15	1% glycerin....	15,500,000	19,500,000	23,500,000	**25,500,000**
3....	Nov. 22	1% glycerin....	....	5,000,000	6,700,000	**7,000,000**
4....	Nov. 23	0.5% glycerin...	63,000,000	**76,000,000**	68,000,000	72,000,000
5....	Nov. 23	0.5% glycerin...	12,000,000	14,500,000	19,000,000	**19,000,000**
6....	Dec. 2	1% glycerin....	16,000,000	25,000,000	28,000,000	**30,000,000**
7....	Dec. 2	1% glycerin....	10,500,000	16,000,000	16,500,000	**17,000,000**
8....	Nov. 30	1% glycerin....	....	12,000,000	14,000,000	**14,000,000**
9....	Nov. 30	0.5% glycerin...	....	9,500,000	14,500,000	**15,000,000**

* The higher 14-day count in each test is printed in bold-faced type.

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[35] See footnote 22.

were 18° C. and 25° C. A slight advantage seems to be indicated for the 25° incubation, as in all but two of the nine cases the higher count was obtained at the higher temperature. Generally the difference in favor of the higher temperature has been slight, although occasionally (as in test 2) it was of considerable size. It is of especial interest to notice that there has seldom been an appreciable increase in the count during the second week at 25°, which means that if 25° is used, a seven-day incubation is sufficient. In the present work, however, 18° for 14 days was used regularly, because it proved more convenient to use the same temperature for both gelatin and agar, and the differences between the two temperatures were so slight as to be of little consequence. There seems to be no good reason for recommending any special temperature for agar plates, provided it is not too high, and the period of incubation is long enough to allow all the colonies possible to develop.

Another point that proved to be of considerable importance in its influence upon the count was the number of colonies allowed per plate. When using gelatin, preference was given to counts obtained from plates having between 50 and 150 colonies each, as with smaller numbers chance variations would have too great an influence on the count, and with greater numbers the count was too likely to be reduced because of liquefaction or overcrowding. Gelatin plates with as many as 200 or as few as 30 colonies were sometimes used when others were not available, and in many cases they were probably as reliable as those within the narrower limits. Agar plates apparently give reliable counts with as many as 300 colonies per plate. Ordinarily, however, the number of bacteria in soil is so nearly constant that it is easily possible to obtain plates with more than 50 and less than 150 colonies each. The dilutions 1 to 100,000 and 1 to 200,000 were ordinarily used. These two dilutions gave the desired number of colonies per plate, unless the count was under 5,000,000 or over 30,000,000 per gram of moist soil — which rarely happens in field soils. In some special cases in which abnormally high numbers were expected, such as when the soil was frozen, recently aerated or manured, it was necessary to use greater dilutions.

Technical Bulletin No. 38 dealt mainly with the subject of media. It was shown that various media gave very similar counts. If anything, the highest counts were obtained upon a soil-extract gelatin; but an asparaginate agar [36] was especially recommended, principally because of its definite chemical composition. Since that paper was published, further modifications of both these media have been made.

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[36] Distilled water 1000 c. c., agar 12 g., dextrose 1 g., sodium asparaginate 1 g., $NH_4H_2PO_4$ 1.5 g., $CaCl_2$ 0.1 g., $MgSO_4$ 0.2 g., KCl 0.1 g., $FeCl_3$ trace. Reaction adjusted to 1.0 per ct. normal acid to phenolphthalein.

Comparison between soil-extract gelatin and tap-water gelatin.—A few figures given in the paper just mentioned indicated that tap-water might be substituted for soil-extract in the gelatin; but at that time so few results were available that it hardly seemed wise to recommend tap-water gelatin. More recently eleven more tests comparing soil-extract gelatin and tap-water gelatin have been made (Table II). The counts upon the two media were generally almost the same; and the counts on soil-extract gelatin were higher than on tap-water gelatin in only three of the eleven tests. There seems, therefore, to be no reason for preferring the soil-extract formula; and as tap-water gelatin is of simpler composition, it is now recommended.

Table II.—Tests Comparing Soil-extract Gelatin with Tap-water Gelatin.

Test No.	Date.	Soil.	Colonies per gram of dry soil developing on	
			Soil-extract gelatin.	Tap-water gelatin.
	1913.			
1	Sept. 21	Dunkirk fine sand	6,000,000	6,000,000
2	Sept. 21	Id.	5,800,000	**6,000,000**
3	Sept. 24	Ontario fine sandy loam	***22,500,000**	21,800,000
4	Sept. 24	Id.	22,000,000	**24,000,000**
5	Oct. 11	Dunkirk loam	7,000,000	**7,700,000**
6	Oct. 11	Id.	3,800,000	**4,000,000**
7	Oct. 19	Dunkirk silty clay loam	9,000,000	**10,800,000**
8	Oct. 20	Id. (manured)	185,000,000	**245,000,000**
9	Oct. 20	Honeoye stony loam	**26,000,000**	25,500,000
10	Oct. 20	Id.	27,000,000	27,000,000
11	Oct. 22	Dunkirk gravelly loam	**35,000,000**	28,000,000

* The higher count in each test is printed in bold-faced type.

Comparison between different brands of gelatin.— A footnote in Technical Bulletin No. 38 stated that the use of 20 per ct. gelatin, instead of 12 per ct., prevented rapid liquefaction and thus allowed a higher count. Some work has been done since then to determine what concentration of gelatin is best. Besides Gold Label gelatin, which was used thruout the earlier work, two other commercial brands of gelatin have been used: one prepared for bacteriological work by the U. S. Glue Co. of Milwaukee; the other, called "Bacto-gelatin," prepared by the Digestive Ferments Co. of Detroit. Both

TABLE III.—COMPARISON OF DIFFERENT CONCENTRATIONS OF GELATIN FOR PLATING SOIL BACTERIA.

PART I.

Test No.	Date.	Soil.	Colonies per gram of dry soil developing on					
			Gold Label gelatin.				U. S. G. Co. gelatin.	
			12%	15%	18%	20%	12%	8%
	1914							
1	Oct. 23	Dunkirk silty clay loam	21,000,000	....	....	***25,000,000**	....	....
2	Oct. 23	Id	30,000,000	....	....	**31,000,000**	....	....
3	Oct. 23	Id	32,000,000	....	....	30,000,000	....	....
4	Oct. 28	Id	6,800,000	....	....	6,000,000	....	....
5	Nov. 5	Id	17,300,000	....	....	16,500,000	....	....
6	Nov. 5	Id	20,000,000	....	....	**20,500,000**	....	....
7	Nov. 6	Id	20,000,000	....	....	**20,000,000**	....	....
8	Nov. 6	Id	19,000,000	....	....	18,000,000	....	....
	1916							
9	Jan. 27	Dunkirk fine sand	3,900,000	3,400,000	3,300,000	....	3,800,000	....
10	Jan. 27	Id	4,000,000	4,000,000	3,800,000	....	**4,300,000**	....
11	Jan. 28	Honeoye stony loam	†20,000,000	16,300,000	17,500,000	....	**21,500,000**	....
12	Jan. 28	Id	15,500,000	14,000,000	**16,000,000**	....	**17,000,000**	....
13	Jan. 28	Dunkirk sandy loam	3,000,000	2,700,000	1,700,000	....	2,800,000	....
14	Jan. 28	Id	5,200,000	4,300,000	4,200,000	....	4,900,000	....
15	Jan. 29	Dunkirk gravelly loam	27,500,000	22,000,000	26,000,000	....	**32,000,000**	....
16	Jan. 29	Id	22,500,000	(liquefied)	(liquefied)	....	21,500,000	....
17	Feb. 17	Dunkirk silty clay loam	4,000,000	3,500,000	3,200,000	....	**4,600,000**	....
18	Feb. 17	Id	15,000,000	9,000,000	9,200,000	....	9,500,000	....
19	Feb. 18	Id	15,500,000	15,000,000	13,500,000	....	**15,800,000**	....
20	Feb. 18	Id	22,000,000	19,000,000	20,000,000	....	**26,000,000**	....
21	Feb. 18	Id	7,800,000	6,000,000	7,000,000	....	**10,000,000**	....
22	Mar. 2	Id	25,000,000	....	**27,000,000**	....	**29,000,000**	**32,000,000**
23	Mar. 2	Id	22,500,000	....	**23,000,000**	....	**25,500,000**	21,500,000
24	Mar. 3	Id	34,000,000	....	32,500,000	....	32,000,000	32,000,000
25	Mar. 3	Id	31,000,000	....	29,000,000	....	**36,500,000**	28,000,000
26	Mar. 14	Id	84,000,000	....	77,500,000	....	**90,000,000**	80,000,000
27	Mar. 14	Id	17,800,000	....	17,600,000	....	**21,600,000**	**19,800,000**
28	Mar. 20	Id	69,000,000	....	65,000,000	....	**82,000,000**	**†80,000,000**
29	Mar. 20	Id	26,000,000	....	**27,500,000**	....	**32,000,000**	**27,500,000**
30	Mar. 24	Id	23,500,000	....	22,300,000	....	**28,000,000**	**28,000,000**
31	Mar. 24	Id	18,800,000	....	16,000,000	....	**24,000,000**	18,000,000
32	Mar. 25	Id	23,500,000	....	**23,500,000**	....	**31,000,000**	26,000,000
33	Mar. 25	Id	22,500,000	....	**24,500,000**	....	**31,000,000**	**22,500,000**
34	May 27	Volusia silt loam	6,500,000	....	....	....	....	**8,000,000**
35	May 27	Id	12,000,000	....	....	....	....	(liquefied)

PART II.

Test No.	Date	Soil	Colonies per gram of dry soil developing on						
			Gold Label gelatin		U. S. G. Co. gelatin		" Bacto-gelatin "		
			12%	18%	10%	12%	10%	12%	15%
	1916								
36......	Apr. 20	Dunkirk silty clay loam......	15,500,000	**18,800 000**	....	**19,500 00**	**15,500,000**	....	....
37......	Apr. 20	Id..........................	10,000,000	**12,500,000**	....	9,500,000	8,000,000	....	....
38......	May 25	Allis silt loam..............	12,000,000	....	....	**17,000,000**	**17,000,000**	....	....
39......	May 25	Id..........................	11,500,000	....	....	**20,000,000**	11,000,000	....	....
40......	June 10	Volusia silt loam............	7,000,000	**9,000,000**	....	....	....	....	....
41......	June 10	Id..........................	8,000,000	**9,500,000**	....	....	....	....	....
42......	June 6	Id..........................	†12,500,000	**29,000,000**	....	....	....	....	....
43......	June 6	Id..........................	†32,000,000	†22,000,000	....	....	....	....	....
44......	June 23	Dunkirk silty clay loam......	....	32,500,000	30,000,000	38,000,000	....	32,000,000	32,000,000
45......	June 23	Id..........................	33,000,000	**35,500,000**	28,000,000	22,500,000	....	23,500,000	24,500,000
46......	June 24	Id..........................	....	32,000,000	....	20,000,000	....	18,500,000	....
47......	June 24	Id..........................	....	17,000,000	....	12,000,000	....	9,000,000	....
48......	June 26	Id..........................	(liquefied)	4,500,000	....	4,700,000	....	3,750,000	4,000,000
49......	June 26	Id..........................	†18,000,000	16,500,000	16,300,000	16,000,000	....	15,500,000	17,000,000
50......	June 28	Id..........................	†7,000,000	**9,000,000**	**9,000,000**	**8,600,000**	....	**8,500,000**	**9,500,000**
51......	June 28	Id..........................	†12,000,000	....	**14,000,000**	**16,000,000**	....	....	**15,000,000**
52......	June 28	Id..........................	....	....	9,500,000	9,200,000	....	....	9,200,000

* Counts as high or higher than the corresponding counts on 12 per ct. Gold Label gelatin are printed in bold-faced type.

† These counts are inexact because of rapid liquefaction.

of these brands of gelatin have a higher jellying power than Gold Label gelatin; so that if they are made up in a strength of 10 per ct. about as firm a jelly is formed as with 15 per ct. Gold Label gelatin.

The results of this work are given in Table III. In Part I of this table, various concentrations of Gold Label gelatin are compared with two different concentrations of U. S. Glue Co. gelatin; in Part II, 15 per ct. and 20 per ct. Gold Label gelatin are omitted, but three strengths of Bacto-gelatin are included. The omission of 20 per ct. Gold Label gelatin in the latter part of the work was simply because it did not allow colonies to become large and hence did not favor qualitative work. From a quantitative standpoint, however, it has made a very good showing, as it gave a higher count than 12 per ct. Gold Label gelatin in three of the eight tests and an equally high count in one of the others. Its use is highly to be recommended if quantitative results alone are desired, as its slow liquefaction makes a long incubation possible even if a temperature as low as 18° C. is not available. Eighteen per ct. Gold Label gelatin was given a more thoro test merely because it was better for qualitative work, as will be discussed later (p. 114).

Of all the different formulae in Table III, the best quantitative results were obtained with 12 per ct. U. S. Glue Co. gelatin. It has given a higher count than Gold Label gelatin in 25 of the 38 times when a comparison has been made between the two media. Its only disadvantage was for qualitative purposes, as it did not allow certain Actinomycetes to show up as distinctly or with as great chromogenesis as on either of the other two brands of gelatin. Ten per ct. U. S. Glue Co. gelatin was tested only six times, but in those six tests, it gave as good results as did the 12 per ct. formula.

Bacto-gelatin has made a rather poorer showing in these tests as it has equalled the count on Gold Label gelatin in only five of the fourteen tests. This apparent inferiority, however, may be due to the fact that this gelatin is so purified at the factory that it does not need to be clarified with white of egg, and the materials contained in the white of egg may serve to increase the development of bacteria on media to which white of egg has been added. In that case, it ought to prove possible to find some substance of definite chemical composition which could be added to Bacto-gelatin in the place of white of egg; and thus its apparent inferiority would prove actually a point of superiority. No such substance has been found as yet, and the tap-water formula has proved the best thus far investigated.

As a result of these tests, the following formula is recommended as a gelatin medium for quantitative purposes:

Tap-water, 1000 c. c.
Gelatin 200 g. of Gold Label gelatin.
or 120 g. of Bacto-gelatin or of U. S. G. Co. gelatin.
Reaction adjusted to 0.5 per ct. normal acid to phenolphthalein. (This will require about 20–30 c. c. normal NaOH for Gold Label gelatin, but under 10 c. c. with either of the other brands.)
Clarified with white of egg in case Gold Label gelatin or U. S. Glue Co. gelatin is used, but unclarified if Bacto-gelatin is used.

Comparison between plain asparaginate agar and asparaginate agar with glycerin.—The asparaginate agar (formula given in footnote No. 36), has been modified by the addition of 10 grams of glycerin per liter. This was found to favor the Actinomycetes, to stimulate chromogenesis and to increase the size of the colonies to such an extent that it was immediately recognized as an improvement provided it gave a count as high as the unmodified formula. The 25 tests given in Table IV were made to investigate this point. In these 25 tests, the asparaginate-glycerin formula has given a higher count than the unmodified formula in 12 cases and the unmodified formula has given the higher count in 11 cases. As the difference between the two counts has never been great, this seems to indicate that one formula gives as good quantitative results as the other. The two formulae have also been compared in an indirect way, with results that distinctly favor the asparaginate-glycerin medium. In Table IX of Technical Bulletin No. 38, 96 comparisons between gelatin and plain asparaginate agar were given. In only 34 of these cases was the count higher on the asparaginate agar than on gelatin. Add to these results the data given in Table IV of the present paper and we have 121 tests in all, in 55 of which the count was higher on the unmodified asparaginate agar than on gelatin. Now in Table V are listed 72 comparisons between gelatin and asparaginate-glycerin agar, to which are to be added the 25 comparisons given in Table IV. In these 87 tests, a higher count has been obtained upon the asparaginate-glycerin formula in 67 cases, a considerably better showing than that made by the unmodified formula. It seems well, therefore, to recommend the glycerin formula, because it gives as good or better counts than the unmodified formula and allows greater differentiation between the colonies of the different kinds of bacteria.

Comparison between gelatin and agar.—Whether to use this asparaginate-glycerin agar or the tap-water gelatin will depend upon the conditions under which the work is being done. The data given in tables IV and V show that the agar gives, if anything, rather higher counts than the gelatin, but does not do so consistently enough to make its use advisable in all cases. The counts obtained upon the two media are nearly enough alike so that if a medium for quantitative work alone is desired, the choice between them should rest upon other considerations such as whether low temperatures are available for incubation. If, however, qualitative results are desired, each medium has distinct advantages of its own. In this case a number of other factors besides the total count must be taken into consideration. A discussion of them is given in the following pages.

TABLE IV.—TESTS COMPARING ASPARAGINATE-GLYCERIN AGAR WITH PLAIN ASPARAGINATE AGAR AND WITH GELATIN.

Test No.	Date.	Soil.	Colonies per gram of dry soil developing on		
			Gelatin.	Asparaginate agar.	Asparaginate-glycerin agar.
	1915				
1	Feb. 19	Dunkirk silty clay loam	26,000,000	**†37,000,000**	27,000,000
2	Feb. 25	Id	*44,000,000	**42,000,000**	32,000,000
3	Feb. 25	Id. (sub-soil)	‡30,000,000	**47,500,000**	41,000,000
4	Mar. 10	Id. (surface soil)	18,000,000	**32,000,000**	21,000,000
5	Mar. 10	Id. (sub-soil)	5,000,000	**8,800,000**	5,000,000
6	Mar. 11	Id. (surface soil)	‡26,000,000	**34,000,000**	25,000,000
7	Mar. 11	Id. (sub-soil)	11,000,000	**20,000,000**	11,000,000
8	Mar. 13	Volusia silt loam	24,500,000	28,000,000	28,000,000
9	Mar. 13	Id	‡13,000,000	**22,000,000**	16,000,000
10	Sept. 8	Dunkirk silty clay loam	11,500,000	11,500,000	**14,500,000**
11	Sept. 8	Id	19,500,000	**24,500,000**	22,000,000
12	Sept. 16	Id	*25,000,000	24,000,000	24,000,000
13	Sept. 16	Id	27,000,000	27,000,000	**31,000,000**
14	Sept. 16	Id	20,000,000	24,000,000	**26,000,000**
	1916				
15	Sept. 27	Id. (grass roots added)	75,000,000	**97,000,000**	90,000,000
16	Sept. 27	Id. (unmanured)	9,000,000	17,500,000	**19,500,000**
17	Oct. 18	Id	11,000,000	17,000,000	**21,000,000**
18	Oct. 18	Id	11,000,000	14,500,000	**17,000,000**
19	Oct. 18	Id	16,000,000	**23,000,000**	22,500,000
20	Oct. 20	Volusia silt loam	9,500,000	12,500,000	**16,000,000**
21	Oct. 20	Id	10,800,000	11,500,000	**16,000,000**
22	Nov. 11	Id	11,000,000	19,000,000	**19,500,000**
23	Nov. 23	Id	11,000,000	19,000,000	**21,000,000**
24	Nov. 29	Dunkirk silty clay loam	4,600,000	7,900,000	**8,400,000**
25	Nov. 29	Id	8,100,000	9,000,000	**10,000,000**

* These are the only two counts on gelatin which are higher than the corresponding counts on asparaginate agar.

† The higher agar count in each test is printed in bold-faced type.

‡ These gelatin counts are inexact because of rapid liquefaction.

TABLE V.—COMPARISON OF GELATIN WITH ASPARAGINATE-GLYCERIN AGAR.

Test No.	Date.	Soil.	Colonies per gram of dry soil developing on	
			Tap-water gelatin.	Asparaginate-glycerin agar.
	1915			
1	Sept. 21	Dunkirk fine sand	6,000,000	***7,500,000**
2	Sept. 21	Id.	6,000,000	**6,200,000**
3	Sept. 24	Ontario fine sandy loam	22,000,000	**25,000,000**
4	Sept. 24	Id.	**24,000,000**	19,500,000
5	Oct. 11	Dunkirk sandy loam	7,700,000	**14,000,000**
6	Oct. 11	Id.	4,000,000	**6,600,000**
7	Oct. 19	Dunkirk silty clay loam	10,800,000	**13,000,000**
8	Oct. 20	Id. (manured)	**245,000,000**	175,000,000
9	Oct. 20	Honeoye stony loam	25,500,000	**30,000,000**
10	Oct. 20	Id.	27,000,000	**32,000,000**
11	Oct. 22	Dunkirk gravelly loam	28,000,000	**33,000,000**
12	Oct. 22	Id.	†20,000,000	**31,000,000**
13	Oct. 22	Dunkirk silty clay loam (manured)	†400,000,000	**470,000,000**
14	Nov. 9	Id. (unmanured)	10,000,000	**13,000,000**
15	Nov. 10	Id.	**38,000,000**	36,500,000
16	Nov. 10	Id.	28,000,000	28,000,000
17	Oct. 28	Id. (manured)	158,000,000	**165,000,000**
18	Nov. 1	Id.	†250,000,000	**350,000,000**
19	Nov. 4	Dunkirk gravelly loam	21,500,000	**32,000,000**
20	Nov. 4	Id.	30,000,000	**35,000,000**
21	Nov. 4	Dunkirk sandy loam	4,500,000	**8,800,000**
22	Nov. 4	Id.	6,600,000	**9,200,000**
23	Nov. 4	Honeoye stony loam	28,000,000	**34,000,000**
24	Nov. 4	Id.	24,500,000	**27,000,000**
25	Nov. 4	Dunkirk sandy loam	7,500,000	**11,400,000**
26	Nov. 4	Id.	8,300,000	**12,000,000**
27	Nov. 5	Dunkirk silty clay loam (manured)	200,000,000	**368,000,000**
28	Nov. 9	Id.	65,800,000	**130,000,000**
29	Nov. 13	Id.	†76,000,000	**145,000,000**
30	Nov. 22	Id.	76,000,000	76,000,000
31	Dec. 7	Id. (unmanured)	9,000,000	**13,000,000**
32	Dec. 7	Id.	4,250 000	**5,750,000**
33	Dec. 7	Id. (manured)	44,000,000	**87,500,000**
34	Dec. 7	Id.	40,000,000	**67,000,000**
35	Nov. 15	Id. (unmanured)	†**32,000,000**	30,000,000
36	Nov. 15	Id.	**22,500,000**	19,500,000

* The higher count in each test is printed in bold-faced type.

† These gelatin counts are inexact because of rapid liquefaction.

TABLE V (*concluded*).

Test No.	Date.	Soil.	Colonies per gram of dry soil developing on	
			Tap-water gelatin.	Asparaginate-glycerin agar.
	1915			
37	Nov. 22	Dunkirk silty clay loam	5,100,000	***5,300,000**
38	Nov. 23	Id	†70,000,000	**76,000,000**
39	Nov. 23	Id	**30,000,000**	23,500,000
40	Dec. 2	Id	**35,000,000**	25,000,000
41	Dec. 2	Id	**16,000,000**	15,800,000
42	Dec. 7	Id	10,000,000	**12,000,000**
	1916			
43	Jan. 27	Dunkirk fine sand	3,600,000	**3,800,000**
44	Jan. 27	Id	4,000,000	**5,500,000**
45	Jan. 29	Dunkirk gravelly loam	**27,000,000**	15,300,000
46	Jan. 29	Id	22,000,000	**23,000,000**
47	Feb. 18	Dunkirk silty clay loam	15,000,000	**16,000,000**
48	Feb. 18	Id	22,000,000	**30,000,000**
49	Feb. 18	Id	7,700,000	**12,000,000**
50	Mar. 14	Id	**84,000,000**	56,000,000
51	Mar. 14	Id	17,800,000	**37,000,000**
52	Mar. 18	Id	**33,000,000**	25,000,000
53	Mar. 18	Id	19,000,000	19,000,000
54	Mar. 20	Id	**69,000,000**	41,000,000
55	Mar. 20	Id	26,000,000	**32,000,000**
56	June 10	Volusia silt loam	9,000,000	**17,000,000**
57	June 10	Id	9,500,000	**12,500,000**
58	May 31	Id	16,000,000	**18,000,000**
59	May 31	Id	†12,500,000	**19,000,000**
60	June 23	Dunkirk silty clay loam	32,500,000	**40,000,000**
61	June 23	Id	**35,500,000**	32,000,000
62	June 24	Id	**32,000,000**	14,500,000
63	June 24	Id	**17,000,000**	9,000,000
64	June 26	Id	**4,500,000**	3,750,000
65	June 26	Id	16,500,000	**17,000,000**
66	June 28	Id	9,000,000	9,000,000
67	June 28	Id	†12,000,000	**18,000,000**
68	Sept. 7	Id	15,000,000	**26,000,000**
69	Sept. 7	Id	8,000,000	**20,000,000**
70	Sept. 8	Id	22,000,000	**27,500,000**
71	Sept. 15	Id	23,000,000	**25,000,000**
72	Sept. 15	Id	10,000,000	**12,000,000**

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* The higher count in each test is printed in bold-faced type.

† These gelatin counts are inexact because of rapid liquefaction.

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PLATING SOIL FOR QUALITATIVE PURPOSES.

Advantages of gelatin.—In making a study of any bacterial flora, it is very important to have a medium that is satisfactory for qualitative as well as quantitative purposes. A perfect medium for qualitative work would be one upon which all the kinds of bacteria present are able to develop, each kind of organism producing a colony easily distinguished from all the other kinds. As a perfect medium is out of the question at present, the best to be hoped for now is one upon which the important soil organisms are able to grow, and on which the colonies of the most common types can be recognized at a glance. If such a medium were available, it would be a fairly easy task to count the number of colonies of each type present, then isolate and study cultures made from the unknown colonies, thus obtaining a satisfactory knowledge of the flora of the soil investigated.

Needless to say, no medium has yet been found that is satisfactory from this point of view. Gelatin has been used most frequently in the present work, because of the ease with which certain common soil forms are recognized by means of their gelatin colonies. Nearly all the spore-forming bacteria common in soil can be recognized on gelatin plates after a little experience. Of the non-spore-formers, certain rapid liquefiers, such as *Ps. fluorescens*, produce very characteristic gelatin colonies. An even greater advantage of gelatin is the ease with which even the most minute colonies of the Actinomycetes can be distinguished (with a low-power microscope) from the colonies of lower bacteria.

The scheme on page 31 will serve as a guide to help in distinguishing the different kinds of bacteria from their gelatin colonies. It must be remembered, however, that only the typical forms of colonies are included in this classification, and nearly all the organisms in question occur frequently in atypical forms.

The best concentration of gelatin to use for qualitative purposes is less than that recommended for quantitative work. The concentration used should vary according to the temperature available for incubation. If 18° C. is available, 12 per ct. Gold Label gelatin is generally satisfactory, or if that liquefies too rapidly, then 15 per ct.; while if a temperature of 20 or 21° must be used, 18 per ct. Gold Label gelatin proves best. The concentration used should be just great enough to prevent the rapid liquefiers from spreading over the entire plate during a seven-day incubation. If the concentration is less than this the plates may be lost through liquefaction; while if it is greater some of the rapid liquefiers are suppressed entirely — which of course gives a false idea as to the relative abundance of the different kinds of bacteria in the soil. U. S. Glue Co. gelatin is not satisfactory for qualitative purposes, because

CLASSIFICATION OF GELATIN COLONIES.

A. 4-day colonies generally over 1 cm. in diameter; liquefaction rapid.
  1. Colonies with rhizoid to filamentous edges. *B. mycoides* Flügge
     (Under certain circumstances, however, *B. cereus* develops colonies of this type.)
  2. Colonies with smooth edges (macroscopically), with a heavy pellicle that is granular and generally concentrically ringed. *B. cereus* Frankland
  3. Colonies with entire edges, structureless, sometimes fluorescent but generally consisting only of a clear liquefied zone, round to amoeboid in shape, and often covering the entire plate by the seventh day. *Ps. fluorescens* (Flügge) Migula
  4. Large liquefying colonies other than the above often occur; but generally belong to the less common spore-formers, or to forms closely related to *Ps. fluorescens*.

B. Colonies seldom over 1 cm. in diameter even on seventh day.
  1. 7-day colonies generally 2-8 mm. in diameter, consisting of a white opaque flocculent center usually surrounded by a clear zone of liquefied gelatin. *B. megatherium* De Bary
  2. Colonies generally punctiform, and only rarely over 2 mm. in diameter. Viewed with low-power microscope, they have entire edges. The non-spore-formers (not including the *Ps. fluorescens* group).
  3. Colonies punctiform to 3 mm. in diameter. Of tough consistency, so that the entire colony can be picked out of the medium without losing its shape. Viewed under low-power microscope, edges are filamentous, radiate. Colonies are often surrounded by a broad zone of brown pigment. Various types of Actinomyces
  4. Some small liquefying colonies without characteristic appearance often occur that are really atypical forms of *B. cereus*, *B. megatherium*, *Ps. fluorescens* or of some of the less common organisms.

the colonies of Actinomycetes cannot be distinguished on it as readily as upon either of the other two brands. Bacto-gelatin may be used with as good results as Gold Label gelatin; but as it has a higher jellying power, a lower concentration must be employed. The best concentration of Bacto-gelatin for qualitative work proves to be about 10 per ct.

Gelatin, however, is far from perfect for qualitative work. This has become especially apparent since the comparative insignificance of the spore-forming bacteria in soil has been realized. Although the different spore-formers can generally be recognized by their gelatin colonies, the other soil micro-organisms (with the exception of the *Ps. fluorescens* group) all produce non-characteristic colonies. The Actinomycetes can be distinguished from the lower bacteria, but not from each other; while all the Bacterium forms (together with a few Coccus and Pseudomonas forms) produce punctiform colonies that look exactly alike. These two groups together constitute about 90 per ct. of all the colonies on the plates. There is actually a great variety of Actinomycetes in soil, and quite likely an equal variety of lower bacteria that produce punctiform colonies on gelatin, all of which cannot be distinguished from each other on gelatin plates; so it is plain that gelatin is not entirely satisfactory for qualitative work.

Advantages of agar.—At present agar seems to be more promising for this purpose than gelatin. No agar medium has been found as yet on which the colonies of the different non-spore-formers can be distinguished from each other; but the asparaginate-glycerin agar (see page 109) is of considerable value in distinguishing the different kinds of Actinomycetes. Unfortunately, however, the types of Actinomycetes that produce the most easily recognized colonies on this medium are generally the less common ones. Two of the three most abundant types produce non-characteristic colonies. There are media upon which all three of these most abundant types produce characteristic growth; but no such medium has been found that gives a high enough count from soil to be satisfactory for plating purposes. Work is now in progress with the object of finding such a medium. When a satisfactory medium for the Actinomycetes is obtained, a similar attempt should be made to find a medium adapted to the non-spore-formers.

Technic at present recommended.—The best procedure at present available for making a qualitative study requires the use of two media for plating. From gelatin the following points can be recognized: number of colonies of *B. mycoides*, *B. megatherium*, *B. cereus* and *Ps. fluorescens*; and also the relative numbers of Actinomycetes and lower bacteria. On asparaginate-glycerin agar it is possible to count the colonies of several types of Actinomycetes. If cultures of the unknown types of Actinomycetes are to be isolated for further study, it is advisable to use the asparaginate-glycerin agar for their selection. If cultures are to be isolated from the punctiform colonies of lower bacteria, they may be selected from either medium.

A long incubation at comparatively low temperatures is so very important in qualitative work that it needs special emphasis. Investigators who have used incubation periods of four days or less in quantitative work have claimed that as it was impossible to obtain a complete count, results obtained from the plates would have only a comparative value at best and that an incubation of three or four days would give as good comparative figures as a longer one. This argument may be justified when plating is done for quantitative purposes alone, but not when qualitative results are desired. Colonies of the more slowly growing organisms just begin to appear in four days. Counts made on the fourth day would include only a very small proportion of the Actinomycetes, and almost none of the large group of slow-growing non-spore-formers. Naturally a qualitative study of soil bacteria based on plates incubated for such a short time would give a very erroneous conception of the actual soil flora.

An example of an error caused in this way can be obtained by comparing the work of Chester[37] and that of Hiltner and Störmer.[38]

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[37] Loc. cit., see footnotes 1A, 2, and 3.

[38] Loc. cit., footnote 4.

Hiltner and Störmer kept their plates until no more new colonies appeared (sometimes as long as ten days); while Chester counted his plates on the fourth day. As stated in the preceding paper (see p. 99), the number of punctiform colonies and of Actinomyces colonies obtained by Hiltner and Störmer agrees very closely with the results obtained in the present work. Chester evidently obtained different results, although it is hard to compare the two pieces of work as the results of each are described in entirely different ways. The chief reason for thinking that Chester's results differed from those of the present investigation in this respect is because only two of his " species " described in Report 11[39] belong to the group which has been found the most abundant in the present work (slowly liquefying or non-liquefying non-spore-forming short rods). Similarly in Report 14[40], where he has described the three predominating micro-organisms in one sample of soil, only the least abundant one of the three is a non-spore-former, and that one is motile so that it does not belong to the group which has been found most abundant in all the soils studied in the course of the present work. The natural inference is that Chester's short period of incubation caused him to overlook the most numerous group of bacteria in soil.

THE STUDY OF PURE CULTURES.

The classification card of the Society of American Bacteriologists.—Including the work of the present series of bulletins and that published in Cornell Bul. 338,[41] over a thousand cultures have been isolated for study. In the Cornell work all of the cultures were studied by means of the methods called for by the classification card of the Society of American Bacteriologists. To some extent the same method of study has been used in the later work, and as the classification card represents the best attempt ever made to standardize descriptions of bacteria, a brief description of it is necessary here. A more detailed description of the card was published some time ago by Harding,[42] and more recently by Rahn and Harding.[43]

This classification card is designed as a means of recording in a uniform manner the important characteristics of different cultures of bacteria. One card is to be filled out for each culture studied. Under each heading on the card is a series of descriptive terms of which the one applying to the culture in question is to be underlined. The characteristics included on the card (in the order of the amount of stress laid upon them) are: (1) Important morphological characteristics such as shape, spore-production, presence and arrangement of flagella; (2) physiological characteristics, such as liquefaction of gelatin, production of acid, reduction of nitrates, and so forth; (3) less important morphological characteristics, such as size, structure, and grouping; (4) cultural characteristics, such as form of growth in broth, on agar and so forth.

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[39] Loc. cit., footnote 2.

[40] Loc. cit., footnote 1A.

[41] Loc. cit., footnote 20.

[42] Harding, H. A. The constancy of certain physiological characteristics in the classification of bacteria. N. Y. Agr. Exp. Sta., Tech. Bul. 13. 1910.

[43] Rahn, O., and Harding, H. A. Die Bemühungen zur einheitlichen Beschreibung der Bakterien in Amerika. *Centbl. Bakt.*, II Abt., 42:385–393. 1914.

The group number.—As a means of simplifying the description of these characteristics and the classification of the organisms, those features that are considered of greatest diagnostic value are summed up in what is called the "group number." In the group number, the symbol of the genus according to Migula is followed by a series of ten digits, each of which denotes the presence or absence of some characteristic. The group number is as follows:

100.	Endospores produced
200.	Endospores not produced
10.	Aerobic (Strict)
20.	Facultative anaerobic
30.	Anaerobic (Strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.04	No growth with lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.004	No growth with saccharose
.0001	Nitrates reduced with evolution of gas
.0002	Nitrates not reduced
.0003	Nitrates reduced without gas formation
.00001	Fluorescent
.00002	Violet chromogens
.00003	Blue chromogens
.00004	Green chromogens
.00005	Yellow chromogens
.00006	Orange chromogens
.00007	Red chromogens
.00008	Brown chromogens
.00009	Pink chromogens
.00000	Non-chromogenic
.000001	Diastasic action on potato starch, strong
.000002	Diastasic action on potato starch, feeble
.000003	Diastasic action on potato starch, absent
.0000001	Acid and gas from glycerine
.0000002	Acid without gas from glycerine
.0000003	No acid from glycerine
.0000004	No growth with glycerine

The genus according to the system of Migula is given its proper symbol which precedes the number thus:

Bacillus coli (Esch.) Mig. becomes B. 222.111102.

Value of the card in studying soil bacteria.—It was hoped at the outset that each type of soil micro-organism would have a constant and distinctive group number. If that were the case, a description of the different organisms would prove a very simple task. It was realized, however, even before the preliminary work given in Cornell Bul. No. 338 was ready for publication that the types distinguished by the tests of the classification card were not species—a fact which was thought to be particularly true in the case of the Actinomycetes. It was stated in that bulletin that the descriptions given must be considered to apply to mere physiological types; but it was not realized then how far from satisfactory these types were. Further study has shown that many of the tests included among those on the card and used for diagnosis in the earlier work give too inconsistent results to serve as a basis of distinction; while on the other hand, there are many distinctive characteristics of soil bacteria not mentioned on the classification card. The card has proved of considerable value in the study of those organisms that grow well in liquid media such as the spore-formers and *Ps. fluorescens*; but even with these bacteria different group numbers do not always indicate different species, so it is necessary to know which of the features give consistent and which inconsistent results. In the study of those organisms which grow poorly in liquid media, such as the Actinomycetes and the majority of the non-spore-formers, the card has proved almost valueless.

These facts have made it necessary to develop methods entirely different from those used in the first part of the work. Even in studying the spore-formers, it has been found advantageous to lay less stress on physiological characteristics and much more stress on features of morphology than are called for on the classification card. In studying the Actinomycetes, it has been found necessary to lay most stress upon the appearance of their growth and their color reactions on various special agar media not even mentioned on the classification card. In studying the non-spore-formers, the card has proved of but little value and no other system of classification has yet been worked out.

The changes in technic that have proved necessary in order to obtain a better classification than that given in Cornell Bulletin No. 338 have resulted in changing the number of types recognized. The types of spore-formers are changed less than either of the other two groups. About the same number of types of spore-formers are recognized now as were mentioned in that bulletin; but the ones that are recognized now are much more clearly defined. Many more types of Actinomycetes are recognized now than were when Cornell Bulletin No. 338 was published, although most of them are still too indefinitely defined to be called species. The non-spore-formers, on the other hand, of which over twenty types were listed in that bulletin, have now been gathered together into a few groups. Each of these groups of non-spore-formers may well contain a large number of species; but in the absence of diagnostic features no smaller groups than these can be recognized.

Diagnostic value of the tests given on the card.—The value of the classification card depends upon the consistency of the results obtained in making the tests which it calls for. In the course of the present work considerable information has been obtained to show which of these tests give the most consistent results and are therefore of most value for diagnostic purposes. It has been found that the constancy of the results depends largely upon the methods used in making their determination. The methods generally recommended for making these tests are those given in the second edition of the Standard Methods for the Examination of Water and Sewage.[44] In some cases these methods have been found as satisfactory as any investigated, but often quite different methods were found to give more consistent results.

The information on this subject that has been obtained in the course of this work may be of value to others wishing to use the classification card. It seems worth while, therefore, to take up one by one the various tests that must be made in determining the group number of an organism (together with one or two of the other important tests given on the classification card), and to discuss the methods used as well as the consistency of the results obtained.

Shape of organism. There is a fairly sharp line between the rods of the Bacteriaceae and the round forms of the Coccaceae. Although there are some organisms on the border line between these two forms, there is ordinarily no trouble, in the case of soil bacteria, in distinguishing between rods and cocci.

Size of organism. In a general way, size is of importance, but the limits of variation for any particular organism are quite large. The difference between the large spore-forming bacteria and the small non-spore-formers is striking enough to be of undoubted diagnostic value (even though an occasional spore-former is of very small size). When an organism produces spores, the size of the spores is of greater diagnostic value than the size of the vegetative forms. But for the fact that it is difficult for two different men to make measurements that agree, it would be possible to characterize several of the spore-formers almost entirely by means of the size of their spores.

Flagella. The importance of this point arises from the fact that the Society of American Bacteriologists uses the nomenclature of Migula, and in Migula's classification peritrichic rods are named Bacillus, rods with polar flagella Pseudomonas, and immotile rods Bacterium. If this classification is used, it is important to have some means of determining the arrangement of flagella. Migula's classification, however, is open to considerable criticism, as pointed out by Lehmann and Neumann,[45] because it separates species that are known to be closely related, such as *B. subtilis* and the anthrax organism, or *Bact. aerogenes* and the colon organism. It is generally considered that the type of the genus Bacillus should be *B. subtilis*, but whether the genus should contain all peritrichic rods regardless of spore-production or all spore-producers regardless of motility is a matter of dispute.

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[44] Published by the American Public Health Association. 1912. Boston, Mass.

In the present work, fortunately, it has not been necessary to take either side of this dispute, as the only peritrichic organisms found to be typical of soil are also spore-formers and so belong in the genus Bacillus without question. Lehmann and Neumann, who adopt the classification depending upon spore-production, disregard Migula's genus Pseudomonas; but there seems to be no reason for doing this even if Bacillus is to be characterized by the presence of spores. Rods with spores and polar flagella are rare, if they occur at all, so the genus Pseudomonas can be recognized without regard to the matter of spore-production. It has been distinguished from Bacterium in the present work; but because of the difficulty of determining the presence of flagella, their absence has never been considered of sufficient diagnostic value to distinguish between two cultures that agreed in everything else.

Presence of spores (represented by the first figure in the group number). This has proved a point of considerable diagnostic value. Migula, it is true, claimed that the ability to produce spores is easily lost, and that failure to produce them under ordinary laboratory conditions does not indicate that they are never produced. For this reason Migula lays less stress upon this characteristic than upon flagellation. Altho there is considerable truth in this contention of Migula's, it is coming to be felt that the production of spores is more nearly correlated with other important characteristics than is motility.

It has ordinarily proved fairly easy to determine spore-production. An ordinary dried microscopic preparation of a culture two days old, stained, without heating, by means of either fuchsin or methylene blue, is usually sufficient to show whether spores are present in any particular culture. Occasionally some large rod-shaped organism has been found that does not show spore-production when examined in this way. Some of these cultures, after continued observation for weeks or months, have at last been observed to produce spores and have been identified as *B. simplex* — which is never a vigorous spore-producer. It is believed that the others are also closely related to *B. simplex*, as non-spore-formers are seldom large rods (i. e. over 0.8 microns in diameter). Except in such a case as this, the presence or absence of spores in a microscopic preparation has always proved of great diagnostic value.

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[45] Lehmann, K. B., and Neumann, R. O. Atlas und Grundriss der Bakteriologie. 1912. Munich. See part II, p. 155.

The shape of spores, when they are present, is of equally great diagnostic value. This was pointed out by Meyer and by Gottheil[46] and has been used by Chester[47] and by Ford[48] as well as by other bacteriologists who have recently worked on the spore-forming organisms. Altho the spores present in any preparation vary greatly in shape and size, it is usually possible to recognize some one particular shape and size as typical of the organism in question; and for this reason much stress is laid on that point in the following paper on the spore-formers of soil.

Growth in the absence of oxygen (second figure of the group number). This is a feature of great diagnostic value; but it is not yet known what method to use to obtain the most consistent results. No organism is known to grow in the absence of free oxygen unless some ready source of combined oxygen, such as sugar or nitrate, is at hand. Some organisms which grow anaerobically under certain conditions may possibly be unable to obtain their oxygen from any of the compounds ordinarily used in bacteriological culture. The method used in this work has been to notice the presence or absence of closed-arm growth in fermentation tubes of various sugars. There are two serious objections to this method: First, oxygen may be dissolved in the medium and furnish aerobic conditions even in the closed arm; and secondly, when gas is produced, the bubbles as they form may carry the bacteria up with them and cause turbidity in the closed arm although no actual growth occurs there. The first objection was not considered very important, however, because it was largely obviated in the present work by the use of freshly sterilized media; while the second objection applies only to gas-producing organisms, none of which were studied in this work. For these reasons the fermentation tube method proved fairly satisfactory.

Liquefaction of gelatin (third figure of the group number). This has been determined in stab culture, keeping the tubes under observation for six weeks. It is undoubtedly a feature of considerable diagnostic value, altho there is some possibility that certain kinds of bacteria vary in their ability to liquefy gelatin.

Fermentation of sugars (first three places after the decimal point in the group number). This is a matter that has given very inconsistent results. It is very seldom possible to obtain exactly the same fermentation reactions for the same culture upon repetition of the tests. This may be due to variation in the acid-producing power of the organisms, or possibly to imperfections in technic. The irregularity in results may be due in part to the fact that many of the organisms studied do not grow well in broth. For this reason, the technic was sometimes modified by using litmus-agar containing the sugar in question. " Slant " cultures in such agar often gave better results than broth cultures. In studying the spore-formers, the fermentation reactions were found to be of some value if taken in conjunction with other tests.

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[46] Loc. cit., footnotes 6 and 7.

[47] Loc. cit., footnote 5.

[48] Loc. cit., footnote 10.

Nitrate reduction (fourth figure after decimal point in group number). This test has proved of considerable value in the separation of species, altho sometimes the results appear to be erratic. The erratic results, however, all seem to occur in the case of organisms that grow poorly in nitrate broth. In interpreting the test, therefore, it must be borne in mind that no growth or poor growth in nitrate broth does not necessarily mean that the organism in question is unable to reduce nitrates. This point has already been emphasized by Breed.[49]

Chromogenesis (fifth figure after decimal point). Chromogenesis has proved to be quite constant in the case of the organisms investigated. It is a particularly valuable aid in the classification of Actinomycetes; for altho these organisms are usually gray to brown on ordinary media, the colors produced upon special media are both striking and varied. Excluding the Actinomycetes, there are only two common types of chromogenesis found among soil bacteria. One is the production of fluorescence, the other of a lemon yellow pigment. A few strains have been found that produce an orange pigment. Fluorescence, when present, is of distinct diagnostic value; but the power of producing it is so easily lost that its absence is of no great significance. The production of yellow or orange pigment is constant so far as has been observed.

Diastatic action on potato starch (sixth figure after the decimal point). This may be a constant characteristic, if a satisfactory technic for determining it were known. This determination was added to the group number by the committee that revised the card in 1907; but no official method for making the determination was given. As a result, this figure of the group number seems to be quite generally ignored. In the early part of the present work, some attempt was made to include this determination. The cultures were inoculated onto potato plugs and incubated for two weeks; then the material was crushed in water and a weak solution of iodine added, drop by drop, recording the diastatic action as " strong " if no color change occurred, as " weak " if a reddish color appeared, or as absent if the color was blue. This method soon proved so obviously unsatisfactory that it was discontinued, and the determination has not been used in this classification; hence the question-mark in the group numbers given in the following papers.

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[49] Breed, R. S. The standard method of determining nitrate reduction. *Sci.*, N. S., 41:661. 1914.

Fermentation of glycerin (last figure of group number). This determination has proved of no value whatever. Results are more erratic than in the case of fermentation of sugars.

Production of indol. This test and the characteristics discussed in the two following paragraphs are not necessary in determining the group number; but they are so often included in descriptions of bacteria that a discussion of them here is not out of place.

In the first part of the present work, the determination of indol-production was always made; but it proved so irregular that it was soon discontinued. The method of determining it was to inoculate peptone solution and incubate ten days. It is quite possible that the irregular results may have been due to irregularities in the composition of the peptone itself.

Action on milk. Cultures in plain milk and in litmus milk were made in the first part of the work. Like the indol test, this soon proved to give too inconsistent results to be of much service in classification.

Form of growth. To a considerable extent this is constant and is in some cases a point of considerable diagnostic value. The long chains formed by *B. cereus* and *B. mycoides* cause these organisms to grow in a very different manner from others that break up quickly into isolated rods. The parallel chains of *B. mycoides*, with their false branching, give rise to its characteristic rhizoid growth. These peculiarities of growth impress themselves to a certain extent upon colonies, liquid culture, and growth in stab and on streak. But the limits of variation of the different organisms must be known before this characteristic can be used for diagnosis.

The form of growth produced by Actinomycetes is strikingly constant. Certain characteristics such as consistency, lustre, wrinkling, and presence of aerial hyphae vary so little on the same medium as to be of great diagnostic value.

Reasons for the inconsistent results.— It will be readily seen that such lack of consistency as has just been shown in the results of the various determinations lessens their value for diagnostic purposes. It is possible, even tho not probable, that the inconsistency may be due to actual variations in the physiology of the organisms studied. This possibility is taken into account in compiling the Standard Methods of the American Public Health Association, in which it is recommended that all bacteria be invigorated before study, so that they might all be in an equally vigorous condition. The method of invigoration called for is to inoculate into three successive daily transfers of broth, incubated at 37° C. This procedure was out of the question for nearly all the soil bacteria, because they grow poorly or not at all in broth, and the temperature recommended is an unfavorable one. In an attempt to improve the technic for soil organisms, they were invigorated on agar " slants " at 25° C. Under such conditions all the organisms studied grow vigorously;

but the change in technic did not increase the consistency of the results. The conclusion drawn from this is that the inconsistency in the results generally arose from imperfections in the technic in making the tests rather than from physiological variations of the bacteria.

CONCLUSIONS.

Methods for use in bacteriological investigations of soil are still in an experimental stage. Various methods have been suggested for different purposes, but none of them have been generally adopted. For the purpose of making a flora study of soil, it has proved necessary to develop many new methods.

The poured plate of agar or gelatin has been used as the basis of the present flora studies. Plate culture is useful for two purposes: It gives some idea as to the number of micro-organisms in the soil; and it serves as a basis for qualitative work.

When using the plate method for quantitative work, the important points in technic are the temperature used for incubation and the length of time the plates are incubated. Fairly low temperatures (18° C. for gelatin and not over 25° C. for agar) and at least 7 days incubation have been found to give the best results. The exact composition of the medium used (within certain limits) does not seem to have much influence upon the count obtained. The two media found most satisfactory have been 20 per ct. Gold Label gelatin (or 12 per ct. of either Bacto-gelatin or U. S. Glue Co. gelatin) dissolved in tap-water, and an asparaginate agar with glycerin. Very similar quantitative results, however, can be obtained with the use of other media.

When using the plate method as the basis of qualitative work, the composition of the medium is of much greater significance. No single medium has been found upon which all kinds of soil micro-organisms can be recognized by means of their colonies. The spore-forming bacteria can be recognized fairly easily upon 15 to 18 per ct. Gold Label gelatin (in tap-water). Some kinds of Actinomycetes can be recognized by means of their colonies on asparaginate-glycerin agar; but unfortunately this medium fails to show any difference between two of the most common types. The different kinds of non-spore-formers cannot be recognized upon any of the media yet investigated — with the exception of *Ps. fluorescens*, which is not one of the most abundant types.

For a complete qualitative analysis of the soil flora, at least three media may prove necessary in plating, one of them adapted to bring out the distinctive characteristics of the common soil types of each of the three groups of soil micro-organisms. By plating soil on three such media, it would then be possible to recognize at a glance the most abundant types. The types that could not be recognized by means of their colonies could be isolated and studied in pure culture.

Pure culture studies of this sort have been made in the course of the present work of about a thousand cultures isolated from soil. Various methods have been used in these studies. The classification card of the Society of American Bacteriologists has proved quite well adapted to the study of the spore-formers; and has proved of value in making a preliminary study of other organisms before learning what special tests were best adapted to them. In studying the Actinomycetes, however, none of the tests included in the card study have proved of value, the most important information having been obtained from a study of their growth on various special agar media containing little or no protein material. No methods have yet been found which prove satisfactory for the qualitative study of the non-spore-formers.

SOIL FLORA STUDIES.*

III. SPORE-FORMING BACTERIA IN SOIL.

H. JOEL CONN.

SUMMARY.

1. Certain spore-forming bacteria are always found in soil, but in comparatively small numbers.

2. The three most abundant spore-formers found in the soils investigated are: *B. megatherium* DeBary, *B. mycoides* Flügge, and *B. cereus* Frankland. *B. simplex* Gottheil occurs in somewhat smaller numbers. Occasionally other spore-forming bacteria have been encountered, but not in large enough numbers to be considered important.

3. In the past, the spore-forming bacteria have been considered important soil bacteria. The technic used in this work, however, shows that they comprise but a small part of the flora and apparently occur in normal soil only in the form of spores. This indicates that they are ordinarily inactive in soil.

INTRODUCTION.

The present bulletin is the third of a series of five papers grouped together under the heading of Soil Flora Studies. The first two papers (I. The General Characteristics of the Miscroscopic Flora of Soil; II. Methods Best Adapted to the Study of the Soil Flora) were published in Technical Bulletin No. 57. They constitute an introduction to the series of papers. The remaining two papers of this series are to be entitled:

IV. Non-spore-forming Bacteria in Soil. (Technical Bulletin No. 59.)
V. Actinomycetes in Soil. (Technical Bulletin No. 60.)

HISTORICAL.

No soil micro-organisms have been studied more frequently or more completely than the group of spore-formers. They are constantly present in soil, and are always found on gelatin or agar plates made from soil, producing the largest and most striking of the colonies that develop on such plates. They grow rapidly on ordinary media and if a short period of incubation is used they are among the most numerous of all the kinds of bacteria developing on the plates.

In Houston's[1] paper mentioned in the preceding bulletin the numbers of the different organisms of this group developing on gelatin plates were determined in so nearly the same way as has been done in the present work that the paper is of a good deal of interest, altho now twenty years old. Houston found four common spore-forming bacteria in his soil, which he called *B. mycoides*, *B. subtilis*, *B. mesentericus*, and a "Granular Bacillus." His descriptions of these organisms are careful enough so that it is possible to conclude that his identification of *B. mycoides* was correct, that his *B. subtilis* was actually *B. cereus*, and his "granular bacillus" *B. megatherium*, while his *B. mesentericus* probably contained a number of different, ill-defined, small-spored organisms. As will be shown later, these findings agree very well with those of the present work.

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[1] Houston, A. C. Chemical and bacteriological examination of soils. Local Gov't Board, An. Rept. of Medical Off., 27:251–296. 1898.

* Reprint of Technical Bulletin No. 58, March, 1917.

Houston, however, states that *B. mycoides* is present in soil both in the vegetative form and as spores, whereas the writer[2] has found them present only in the form of spores. Houston's method of determining whether these organisms were present as spores or as vegetative forms was essentially the same as that used by the writer, with the one exception that after mixing the soil with sterile water Houston allowed the infusion to stand one hour before plating in order to secure a thoro mixture. This one difference in technic makes it impossible to accept Houston's conclusions in this particular, as he gives no proof that none of the spores of *B. mycoides* germinated during the hour in which they were suspended in water.

Except for Houston's paper, there is very little reference in the literature to the relative numbers of the different kinds of spore-formers in soil. In general, literature relating to this group of organisms has been concerned either with their activities or with the taxonomy of these organisms. In view of the present doubt as to whether they occur in soil in vegetative form or as spores, their activity in soil is questionable. For this reason the literature concerning them is of interest from the taxonomic rather than from the practical standpoint, and there seems to be no need of reviewing that which relates to their activities. The taxonomic studies of this group, however, are well worth reviewing; for there is no group of bacteria, except that which includes the colon organism, which has been given such a thoro study from the taxonomic point of view.

The importance of studying the botanical relationships of these bacteria was first emphasized by Arthur Meyer and his students, who laid great stress on the need of accurate measurements and careful descriptions. The first important contribution to the taxonomy of the group was by Gottheil,[3] one of Meyer's students.

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[2] Conn, H. J. Are spore-forming bacteria of any significance in soil under normal conditions? N. Y. Agr. Exp. Sta., Tech. Bul. 51. 1916. *Jour. Bact.*, 1:187–195. 1916.

[3] Gottheil, O. Botanische Beschreibung einiger Bodenbakterien. *Centbl. Bakt* II Abt., 7:430, 449, 481, 529, 582, 627, 680, 717. 1901.

The value of Gottheil's work arises from his thoro study of the bacteria and his painstaking descriptions of their morphology. A few years later Neide[4] published a continuation of Gottheil's work, which he took up where it had been dropped by Gottheil. Neide described about as many species as had been described by Gottheil. Both of these pieces of work were done in the same laboratory by the same methods, and with the cultures of one investigator available to the other. For this reason Gottheil's and Neide's papers may be regarded as two installments of a single investigation. Later a further contribution from Meyer's laboratory was published by Holzmüller;[5] but as his paper was concerned only with *B. mycoides* and certain closely related forms, it is not such an important contribution to the general taxonomy of the spore-forming bacteria.

Almost simultaneously with Neide's publication, an independent piece of work was published by Chester.[6] Chester had obtained Gottheil's cultures as well as others from other sources, and published the results of his study of them. He greatly simplified Gottheil's descriptions, making it much easier for anyone else to follow them, and helped remove some of the previous confusion. Unfortunately, however, Neide's paper appeared at so nearly the same time as Chester's that neither author knew of the other's work, and as a result the two pieces of work do not harmonize. No attempt has been made, until within the last year, to harmonize Chester's work with that of Meyer's students.

The work which has finally brought order into the previous chaos is that of Ford and his collaborators.[7] They obtained a larger number of cultures than had been studied by any previous investigators. Most of these cultures were of their own isolation; but they also had for comparison many cultures obtained from other investigators, from Kral's collection, and from the collection of the American Museum of Natural History. With such a large number of cultures it was possible to study correlations of characteristics and to observe differences to better advantage than had been done previously, and their findings have a correspondingly greater authority. Their descriptions of species, moreover, are so carefully and concisely drawn up that they can be quite readily followed by others. Thanks to this work, future investigators need not be as puzzled over the relationships of these bacteria as past workers have been.

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[4] Neide, E. Botanische Beschreibung einige sporenbildenden Bakterien. *Centbl. Bakt.*, II Abt., **12**:1, 161, 337, 539. 1904.

[5] Holzmüller, K. Die Gruppe des Bacillus mycoides Flügge. *Centbl. Bakt.* II Abt., **23**:304–354. 1909.

[6] Chester, F. D. Observations on an important group of soil bacteria: Organisms related to *Bacillus subtilis*. Del. Agr. Exp. Sta., Rept. 15:42–96. 1904. A review of the *Bacillus subtilis* group of bacteria. *Centbl. Bakt.*, II Abt., 13:737–752. 1904.

[7] Ford, W. W., et al. Studies on aerobic spore-bearing non-pathogenic bacteria. *Jour. Bact.*, **1**:273–320, 493–534. 1916.

CLASSIFICATION.

THE THREE MOST COMMON SPECIES.

Occurrence in soil.—Three types of spore-forming bacteria have been found in all the soils studied and have always been observed on the plates, unless too great dilutions were used. They have been satisfactorily identified with *B. megatherium* DeBary, *B. mycoides* Flügge and *B. cereus* Frankland. In each case the identification has been regarded as correct, because the cultures isolated from these soils agree well with the published descriptions given by Chester and by Ford, and also because when representative cultures were sent to Ford for identification, he assigned to them the same names as those already decided upon by the writer. The constant occurrence of these three species in soil and their greater abundance per gram than the other spore-formers make it well to take them up in greater detail than the others.

Of these three species, *B. megatherium* has proved to be slightly more abundant than the other two, plate counts averaging about 375,000 per gram. Plate counts of *B. mycoides* have averaged about 225,000 per gram, and of *B. cereus* about 180,000. It must be recognized that these are not counts of active individuals, but probably they are fairly accurate counts of the spores. Microscopic examinations[8] of various soils have shown that spores of these organisms seldom occur in clumps or chains, so that probably the individual spores are ordinarily separate from each other when they are poured into the plates. Also these three organisms all grow well in gelatin, so that probably every living spore produces a colony. For these reasons it seems safe to conclude that these three organisms do not occur in normal soil to the extent of more than 1,000,000 per gram.

It is interesting to notice that Houston,[9] who made a similar enumeration of the bacteria developing on gelatin plates, did not find as many colonies of the spore-formers; for of *B. mycoides*, which occurred in greatest numbers, he did not find in any case over 100,000 per gram. Nevertheless the numbers of *B. mycoides* which he reports are more nearly equal to the numbers found in the present work than his total counts are to the total counts in the present work. Considering that *B. mycoides* grows more vigorously on gelatin than most soil bacteria, this suggests that Houston's low counts were due to short periods of incubation. In regard to incubation, he merely states that he counted his plates " at as late a date as the liquefaction of the gelatin or the crowding of the micro-organisms " allowed. This probably was not a very long period, as he apparently employed room temperature for incubation, used ordinary beef-extract gelatin, and generally diluted the soil only 1,000 times; which conditions would certainly have caused crowded, rapidly liquefying plates.

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[8] For the technic used, see: Conn, H. J. The direct microscopic examination of bacteria in soil. *Abstr of Bact.* 1: No. 1. 1917.

[9] See footnote 1.

Much attention has been given in the present work to the methods for distinguishing these different organisms from each other. In the previous publication[10] dealing with the bacteria obtained from a soil at Ithaca, N. Y., the classification of these bacteria was attempted by means of the group number of the Society of American Bacteriologists,[11] and an effort was made to assign specific names

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[10] Conn, H. J. A classification of the bacteria in two soil plats of unequal productivity. Cornell Agr. Exp. Sta., Bul. 338:65–113. 1913.

[11] The group number is as follows:

100.	Endospores produced
200.	Endospores not produced
10.	Aerobic (Strict)
20.	Facultative anaerobic
30.	Anaerobic (Strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.04	No growth with lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.004	No growth with saccharose
.0001	Nitrates reduced with evolution of gas
.0002	Nitrates not reduced
.0003	Nitrates reduced without gas formation
.00001	Fluorescent
.00002	Violet chromogens
.00003	Blue “
.00004	Green “
.00005	Yellow “
.00006	Orange “
.00007	Red “
.00008	Brown “
.00009	Pink “
.00000	Non-chromogenic
.000001	Diastasic action on potato starch, strong
.000002	Diastasic action on potato starch, feeble
.000003	Diastasic action on potato starch, absent
.0000001	Acid and gas from glycerin
.0000002	Acid without gas from glycerin
.0000003	No acid from glycerin
.0000004	No growth with glycerin

The genus according to the system of Migula is given its proper symbol which precedes the number thus:

Bacillus coli (Esch.) Mig. becomes B. 222.111102

For a more complete discussion of the group number see Technical Bulletin No. 57, pp. 33–42. (This report, pages 117–126.)

to the different types determined by this means. This attempt was scarcely successful; for it has since proved that the characteristics represented by the group number are not sufficiently constant to be used for classification. The types to which the names *B. mycoides* and *B. megatherium* were assigned are recognized in this publication as well, but the type called *B. subtilis* at that time proves to be untenable. True *B. subtilis* Cohn has not been found in any of the soils investigated. The cultures to which the name *B. subtilis* was assigned in the earlier work may have been to a large extent cultures of *B. cereus*.

The descriptions of these species published by Ford and associates are so complete and apply so well to the cultures obtained in the present work that it is scarcely necessary to give a detailed description of them here. If the brief descriptions that follow prove insufficient to anyone wishing to use them for diagnostic purposes, it would be well to refer to the detailed descriptions of these recent investigators. The chief point of difference between the present descriptions and those given by Ford and his associates is in the dimensions of the organisms. The writer's dimensions are always slightly greater than theirs. This difference merely illustrates the well-known fact that measurements of bacteria are generally inaccurate, either because of the personal factor involved in measuring them or because of inaccurate instruments of measurement.

The names given to these three organisms are by no means the only ones by which these species are known. Cultures of the same species have been variously named by different investigators, and to trace out their synonymy leads to considerable discussion. Instead of giving a list of synonyms in each of the following descriptions, the reader is referred to the recent paper by Ford and associates, and to the earlier paper by Chester, in both of which the matter of synonomy is thoroly discussed.

B. megatherium DeBary, 1884.—This species is to be distinguished from *B. mycoides* and *B. cereus* by the larger average size of its spores, by its poor growth in liquid media, its failure to grow in the closed arm of fermentation tubes of dextrose broth, and by its comparatively slow liquefaction of gelatin.

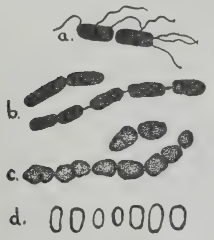

FIG. 1. *B. megatherium.* Magnified 1000 diameters.

Morphology. Very young cultures (under 12 hours) consist of large rods about 1 to 1.5 microns in diameter and about 3 to 6 microns long (Fig. 1, b). They often occur in chains with connecting threads between the rods, resembling strings of sausages. In older cultures the rods generally become swollen and are sometimes full of highly refractive globules (fat drops?). One of the most distinctive characteristics is the presence in cultures a day or more old of large ovoid bodies about

2 by 4 microns in size (Fig. 1, c), which seem to have heavy walls and stain much more lightly than the young rods. Only the young rods are motile, and they are not vigorously so. The flagella are difficult to stain, and the best preparations made show comparatively few flagella on each rod (Fig. 1, a). Spores are formed in the center of the rods, and immediately become free from all trace of the sporangium wall. They are oval to ovoid (or occasionally reniform) and vary considerably in size, from 1.3 to 2 microns in diameter and from 1.5 to 3 microns in length, both extremes often occurring in the same preparation (Fig. 1, d).

Cultural characteristics: Growth in broth floccuent or none, with no surface growth. Gelatin colonies under 10 mm. in diameter, center white, opaque, flocculent or granular, surrounded by a clear liquefied zone. Growth on agar streak cultures smooth, soft, glistening, cream-color, typically with minute drop-like areas of lighter color.

Physiology: The typical group number[12] is B. 111.44420?4. As indicated by this group number, there is ordinarily no growth in sugar and glycerin broths. This does not mean, however, that no acid is produced from sugar or glycerin. Some cultures have produced growth and have acidified one or even all of the sugars. This suggests that the irregularity may be due to the poor growth in liquids. Tested on " slants " of litmus agar, in fact, *B. megatherium* has been found to produce acid from dextrose and sucrose quite regularly. Similarly, its poor growth in broth raises a doubt as to whether the second figure of its group number (denoting it to be a strict aerobe) may be correct; for it grows so poorly even in the open arm of a fermentation tube that its failure to grow in the closed arm does not necessarily prove its inability to grow in the absence of oxygen. It is also possible that its failure to reduce nitrates may be due merely to the fact that it grows poorly in nitrate broth.

It is very doubtful whether this species can be distinguished from *B. petasites* Gottheil. Ford and his collaborators describe both species, stating that they are nearly alike, but may be distinguished by the production of a lemon-yellow pigment by *B. petasites.* None of the cultures obtained in the present work produced any yellow pigment, and were therefore all considered to be *B. megatherium.* It is doubtful, however, whether this characteristic is constant enough in this case to characterize the species; for a culture of *B. petasites* obtained from Ford fails to show the characteristic pigment when cultivated in this laboratory. Ford, Laubach and Rice, in their collection of 518 cultures from soil, found only 32 of *B. megatherium* but 189 of *B. petasites*, which proved to be the most common species in the soils they investigated. It is hardly to be expected that *B. petasites* should be so abundant in one set of soils and entirely lacking in the other. The probable explanation is that cultures Ford would call *B. petasites* were called *B. megatherium* in this work. Chester considers the two forms identical.

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[12] See footnote 13.

(Types 6 and 7 of Cornell Bul. 338 are to be included under this head. These two types, separated on the basis of acid-production, are hardly to be considered distinct.)

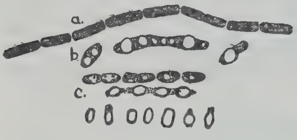

FIG. 2. *B. mycoides.*
Magnified 1000 diameters.

B. mycoides Flügge, 1886. This is the most easily recognized of all the soil bacteria. It can readily be distinguished by its rhizoid growth on agar.

Morphology: Young cultures consist of rods about 0.8 to 1.3 by 2 to 6 microns (Fig. 2, a). They occur in long chains which often lie parallel and show false branching, an arrangement which gives the colonies their rhizoid structure. The very young rods are apparently slightly motile, but no success has been obtained in staining flagella. Gottheil[13] describes several peritrichic flagella. In older cultures, highly refractive globules that do not take ordinary stains (probably fat-drops) appear within the rods, particularly if growing on dextrose agar, sometimes causing the rods to swell to extremely large size and to lose all resemblance to their original form (Fig. 2, b). The lightly stained ovoid bodies that characterize *B. megatherium* have never been observed. Spores are borne centrally and the remnants of the sporangium wall persist for some little time at either end of the spores. Spores are oval to cylindrical, 1.0 to 1.6 by 2 to 2.5 microns, often in fairly long chains (Fig. 2, c).

Cultural characteristics: Growth in broth vigorous, flocculent, with no persistent surface growth. Gelatin colonies rapidly liquefying, filamentous to rhizoid. Growth on agar streak rhizoid, mostly beneath the surface of the medium.

Physiology: The typical group number is B. 121.23230?2. It shows less variation than does the group number of *B. megatherium*, probably because *B. mycoides* grows better in the media used for making the tests. The same acid reactions are obtained in broth culture and on litmus agar.

(This type is the same as Type 1, described in Cornell Bul. 338, and designated *B. mycoides* in that bulletin.)

B. cereus Frankland, 1887.—This type can be distinguished from *B. megatherium* by the smaller size of its spores and by its more vigorous growth in liquid media, and from *B. mycoides* by the absence of rhizoid growth on agar.

Morphology: In morphology it is scarcely to be distinguished from *B. mycoides.* Young rods are 0.8 to 1.3 by 2 to 6 microns, forming long chains (Fig. 3, b); but unlike *B. mycoides* they are very actively motile and are easily shown to be surrounded with numerous flagella (Fig. 3, a). Older rods are often swollen and contain unstained globules (Fig. 3, c). Oval to cylindrical spores, 1.2 to 1.6 by 2 to 2.5 microns, are produced centrally, retain the remnants of the sporangium wall for a short time and often cling together in chains (Fig. 3, d).

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[13] See footnote 3

Cultural characteristics: Growth in broth vigorous, with uniform turbidity, sediment and a surface pellicle. Gelatin colonies quite large, ordinarily round, with entire margin, and covered with a pellicle that generally shows concentric rings, altho under some conditions the colonies are filamentous and resemble those of *B. mycoides*. Growth on agar streak raised, ordinarily rugose, soft to membranous, generally dull; never rhizoid or beneath the surface of the medium like *B. mycoides* or full of clear drop-like areas like *B. megatherium*.

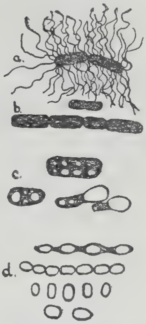

Fig. 3. *B. cereus.* Magnified 1000 diameters.

Physiology: The typical group number is the same as that for *B. mycoides*, B. 121.23230?2, altho considerable variation has been observed, particularly in the production of acid from sugars and from glycerin. In the earlier work, two subtypes were recognized, basing the distinction upon the production of acid from glycerin; but no such distinction is now recognized, because of the inconsistent results obtained in regard to acid production. Dextrose is always acidified by this organism; sucrose is generally acidified; glycerin less frequently, and lactose very seldom. A strain that acidifies lactose has always been found to produce acid from all three of the other compounds. The production of acid from lactose may be a better basis for subdividing the type than acid-production from glycerin. Out of 130 cultures studied, 19 acidified lactose; but it seems unwise to consider them as constituting a separate species, in view of the variation that has been found when cultures have been retested. These 19 cultures agree very well with the description of *B. albolactis* Migula given by Ford and Lawrence.[14]

(This type was not recognized by the methods used when Cornell Bul. 338 was written. Undoubtedly many of the cultures included in Types 2 and 4 should be placed under this head.)

LESS COMMON SPECIES.

Besides the three species present in all of the soils studied, a number of other types have been isolated. Of these, only one, *B. simplex* Gottheil, has been found often enough to deserve special mention here. It is not possible to say whether *B. simplex* occurs in all of the soils investigated, because it does not have a distinctive colony like each of the three more common species and does not occur in great enough abundance to be encountered regularly when chance isolations are made. In a series of chance isolations, *B. simplex* has been found about one-fifth as often as *B. megatherium*, *B. mycoides* or *B. cereus* — a fact which gives some idea as to its frequency in soil. The cultures of *B. simplex* obtained from the soils studied agree fairly well with Chester's and with Ford's descriptions of this species; and the identification has been confirmed by Ford, in the case of a typical culture of this type sent to him.

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[14] See footnote 7.

B. simplex Gottheil, 1901.— This type is characterized by its rather long cylindrical spores, produced only in old cultures (or often not at all), by the occurrence of filaments and long chains of rods, often lightly stained, and by its rather slow growth in gelatin.

Morphology: Young rods are 0.8 to 1.2 by 2.5 to 5 microns (Fig. 4, c). Rods often occur in long chains, and filaments are frequently observed 15 microns in length. In cultures a few days old, these filaments lose their power of staining deeply and become the "shadow forms" characteristic of the species (Fig. 4, b). Young rods are plainly motile; flagella peritrichic, but not very many have been observed on each rod (Fig. 4, a). Spores are seldom produced before the third day and sometimes not until the culture is a week or more old, and are always relatively scarce in comparison with the vegetative forms. Cultures are sometimes obtained that do not produce spores but agree with *B. simplex* in other respects; so that they are thought to be strains of this organism that have lost their power of producing spores, altho without the spores to study it is impossible to be sure of the identity of the organism. The spores are cylindrical, elongate, 0.6 to 1.0 by 1.5 to 3 microns (Fig. 4, d). (It is hard to understand the difference between these measurements and those published by Ford and Lawrence, unless the shape of the spores varies greatly in different strains. The culture sent to Ford and identified by him as *B. simplex* is one that often produces strikingly long spores about 3 microns in length.)

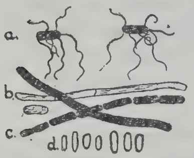

FIG. 4. *B. simplex.* Magnified 1000 diameters.

Cultural characteristics: In broth, slight turbidity and sediment with no surface growth. Gelatin colonies punctiform, slowly liquefying. Growth on agar streak culture, soft, glistening, translucent to almost transparent, with indistinct edges.

Physiology: The typical group number is B. 111.44430?4, the same as that of *B. megatherium* except for the figure referring to nitrate-reduction. As neither *B. megatherium* or *B. simplex* grow well in nitrate broth, it is possible that their lack of agreement in this one respect may have been brought about by irregularities due to their poor growth. Altho, as indicated by the group number, this organism makes no growth in sugar broth, it does grow on litmus agar containing sugars, and it has been found that a vigorously growing culture produces a trace of acid under such conditions from dextrose and sucrose, but not from lactose.

(This type was not recognized at the time when Cornell Bul. 338 was written. The type denoted as "*B. simplex* (?)" in that bulletin was not correctly identified, its actual identity being unknown because the cultures were lost. It is known that some examples of true *B. simplex* were found in that work, as two cultures of it isolated then, are still alive; but at the time that bulletin was published they were not distinguished from the other spore-formers. They were probably included in types 6 and 7.)

Other types.— Besides the four species just described, a number of cultures have been obtained that are plainly different organisms. One or two small-spored organisms have been found that closely resemble *B. mesentericus* (Flügge) Migula; two cultures have been obtained of a round-terminal-spored organism, probably *B. fusiformis* Gottheil; and also several others that have proved difficult to identify. Very little study has been given them, however, and no serious attempt has been made to establish their identity, because they have been found so seldom that they are probably of no importance in soil and, indeed, may have been mere contaminations on the plates, coming from the air or some other source.

Certain spore-formers have been found which are small short rods that grow poorly on ordinary media and may not be closely related to those described above. They have never been found on plates made in the ordinary way. They have occurred only when the soil infusion has been heated to 75° C. before plating. On such plates, presumably, the exclusion of the non-spore forms allows the growth of certain slow-growing spore-producers that are crowded out of the ordinary plates. No study of them has been made, as they are not believed to be very abundant in soil.

GELATIN COLONIES OF THE SPORE-FORMING BACTERIA.

In making a flora study of soil, it is quite important to know the relative abundance of the different types of organisms studied. About the only way to determine this is by means of the relative numbers of their colonies which appear on plate cultures made from soil; and in order to count the colonies of any type, it is necessary that they can be readily distinguished from the colonies of other bacteria. As stated in the preceding paper,[15] gelatin has proved the best medium upon which to recognize the spore-forming bacteria. This is because each of the three most common spore-formers produces a characteristic colony on gelatin (altho *B. simplex* and the other less common types do not).

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[15] N. Y. Agrl. Expt. Sta. Tech. Bul. 57, p. 30.

Unfortunately it is not possible to describe the colonies of these bacteria so that they can be recognized with certainty by other investigators. Differences in technic make so much difference in the appearance of the colonies that the results in different laboratories are likely to be quite divergent. It is possible, however, for one man so to standardize his own technic that he can recognize the colonies of these three spore-formers with some degree of accuracy, after he has once learned their characteristics upon his own plates. The descriptions of the colonies given here may not agree perfectly with those which other investigators may find; but they are given for whatever they may be worth.

It has been found in the present work that all of the colonies on 12 per ct. Gold Label gelatin that have liquefied appreciably (i. e. have become more than one or two millimeters in diameter after seven days at a temperature of 18° C.) are either *Pseudomonas* forms (principally *Ps. fluorescens*) or spore-formers. The *Pseudomonas* colonies are generally structureless, *Ps. fluorescens* producing a rapidly liquefying, almost clear colony, that will liquefy the entire plate if given time. Next to *Ps. fluorescens*, the most rapid liquefier has been found to be *B. mycoides*, which produces large, filamentous to rhizoid colonies. *B. cereus* is almost as rapid a liquefier as *B. mycoides*, and under certain conditions produces colonies that cannot be distinguished from it; but on plates from normal soil, the *B. cereus* colonies are round with entire edges, with a surface membrane containing granules that tend to be arranged concentrically. *B. megatherium* liquefies much more slowly. Its largest colonies are seldom over 1 cm. in diameter. They are characterized by a flocculent center composed of white opaque granules, surrounded by a zone of clear liquefied gelatin. The smaller *B. megatherium* colonies are often under 2 mm. in diameter with no surrounding clear zone, and can be recognized only because of their granular nature, all other punctiform colonies appearing round to the naked eye.

Using these criteria, it has proved possible to obtain a fairly good estimate of the numbers of each of these three species in the different samples of soil investigated. In this way the figures given on p. 6 were obtained. The data recently given,[16] showing that heating the soil infusion did not decrease the number of colonies of spore-formers, depended upon the fact that certain spore-formers produce colonies on gelatin plates that can be easily recognized.

SIGNIFICANCE OF THE SPORE-FORMING BACTERIA IN SOIL.

The great attention that has been given in the past to the spore-forming bacteria of soil is undoubtedly due to their constant presence in soil, their large, striking colonies on agar or gelatin plates, and their rapid growth in laboratory media. Chester,[17] for example, used fairly short periods of incubation, and because of the rapid growth of these organisms, found them to comprise nearly half of the colonies on the plates. Hiltner and Störmer,[18] on the other hand, incubated their plates until no more new colonies appeared (about ten days), and found only 5 per ct. of the colonies to be liquefiers (which category includes nearly all, if not all, of the spore-forming bacteria).

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[16] See footnote 2.

Long incubations, however, have only recently come into general use. The use of short incubations has led to an exaggerated idea of the abundance and importance of spore-forming bacteria in soil. Because of their relative abundance on the plates, they were thoroly studied and it was found that they were vigorous ammonifiers in laboratory culture media. Naturally it was assumed that they were also vigorous ammonifiers in soil. This idea has persisted in spite of repeated observations as to their relatively small abundance. Lipman,[19] for instance, states: "The more important ammonifiers, including members of the *B. subtilis* group and certain Streptothrices, are numerically important in all arable soils." Similarly Stevens and Withers[20] used cultures of *B. subtilis* for inoculating different soils in order to determine their ammonifying capacity, thus very evidently making the assumption that *B. subtilis* is a typical and important soil ammonifier. These two cases are mentioned merely by way of illustration. Writers have frequently made similar statements; and still other writers, who with more caution have refrained from stating that the spore-forming bacteria actually cause ammonification in soil, have referred to their ammonifying powers and left the reader to assume that such must be their activity in soil.

At the present time there is even less justification for such statements than before. It was recently pointed out by the writer[21] that in normal soil — even tho a large quantity of fresh horse manure had been added — there was no evidence of the spore-forming bacteria being present in vegetative form. Since writing that paper, further information on the subject has been obtained by means of the microscopic examination of soil; and it has entirely corroborated this conclusion.

The recent work of Ford and associates,[22] indeed, makes it doubtful if these bacteria should be considered soil organisms in any true sense. These writers found practically the same kinds of spore-formers not only in milk, in water, and in soil, but also in dust, and the species found most frequently in soil were to a striking extent the most abundant forms in milk, dust, and water as well. No one would suggest for a moment that their results indicate that these bacteria are active in dust. The probable explanation is that their spores are omnipresent, and become active only when conditions are favorable.

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[17] Chester, F. D. Study of the predominating bacteria in a soil sample. Del. Agr. Exp. Sta., Rept. 14:52–63. 1903.

[18] Hiltner, L., and Störmer, K. Studien über die Bakterienflora des Ackerbodens. *Kaiserl. Gesundheitsamte, Biol. Abt. Land- u. Forstw.*, 3:445–545. 1903.

[19] Lipman, J. G. In Marshall's Microbiology (2nd edition), p. 325.

[20] Stevens, F. L., Withers, W. A., et al. Studies in Soil Bacteriology III. *Centbl. Bakt.* II Abt., 25:64–80. 1909.

[21] See footnote 2.

[22] See footnote 7.

Just what conditions do favor the growth of these bacteria, it still remains to be learned. *B. mycoides* and *B. cereus* seem to grow best in liquids of high organic content, but the other spore-formers have not been observed to grow rapidly under laboratory conditions except on the surface of agar. It is not impossible that the reason why these species have developed the power of producing spores is because the conditions necessary for their growth are so rarely met in nature that their continued existence would be impossible without some resting stage. In soil, conditions favorable to their growth probably occur just often enough so that the spores do not diminish in number; and when conditions are unfavorable to them, the organisms are inactive.

SOIL FLORA STUDIES.*

IV. NON-SPORE-FORMING BACTERIA IN SOIL.

H. JOEL CONN.

SUMMARY.

1. The majority of colonies on culture plates made from soil are those of non-spore-forming bacteria.

2. The only non-spore-former that has been successfully identified with previously described species is ***Ps. fluorescens*** (Flügge) Migula. This organism is most abundant in freshly aerated soil or in soil to which organic matter has been recently added. It is known to cause ammonification in pure culture, and is therefore thought to take part in the decomposition of organic matter in soil. There are other liquefying non-spore-formers in soil that seem to be closely related to ***Ps. fluorescens.*** They increase in numbers under much the same conditions and are thought to have similar functions.

3. The great majority of the asporogenous bacteria of soil grow so poorly in ordinary laboratory media and produce such small non-characteristic colonies on culture plates that little is known about them. One of the greatest present needs for a soil flora study is the development of methods by which the different species in this group may be identified.

4. The numbers of the non-spore-forming bacteria in soil show greater fluctuations than do those of spore-forming bacteria or of Actinomycetes (the two other large groups of soil bacteria). High numbers of non-spore-formers have been especially noticed in freshly aerated and in freshly manured soil. This indicates that they are among the most active soil micro-organisms.

INTRODUCTION.

In the first paper of this series[1] (p. 99), the bacteria of soil were classified as follows:

- Spore-producers;
- Non-spore-producers:
    - Liquefaction rapid;
    - Liquefaction slow or none:
        - Rods:
            - Yellow chromogenic;
            - Non-chromogenic;
        - Cocci;
- Actinomycetes.

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[1] Conn, H. J. General characteristics of the soil flora. N. Y. Agr. Exp. Sta., Tech. Bul. 57:7–17. 1917.

* Reprint of Technical Bulletin No. 59, March, 1917.

The second paper[2] dealt only with the methods by which these organisms had been studied. The third paper[3] took up the best known altho the least numerous of the three large groups of soil micro-organisms: the spore-forming bacteria. The two which remain to be considered, the non-spore-forming bacteria and the Actinomycetes, are much more numerous in soil than the spore-forming varieties; but much less is known about them.

It has so far proved impossible, indeed, to distinguish the different kinds of organisms in these two groups from each other, or to obtain definite knowledge as to their function in soil. For this reason the present paper on non-spore-forming bacteria and the following one on Actinomycetes are merely preliminary. Even less is known about the non-spore-forming bacteria than of the Actinomycetes; so the present paper can do no more than point out the lines along which their study must develop in the future. Its object is primarily to call attention to the probable importance of non-spore-forming bacteria in soil. It is hoped that this preliminary work may be followed by a more complete study, not only at this Station, but by investigators working elsewhere.

HISTORICAL.

Spore-forming bacteria and Actinomycetes have been studied considerably in the past; but except for the nitrifiers and some of the other organisms concerned in the transformations of nitrogen, scant attention has been given to any non-spore-forming bacteria found in soil. As an illustration of this fact, mention may be made of Löhnis' review of the subject in his "Handbuch der landwirtschaftlichen Bakteriologie." In his section (p. 514) entitled: "Allgemeines über die im Boden vorkommenden Arten von Mikroorganismen" he refers to non-spore-forming bacteria in just two sentences. In these two sentences he says that except for the fluorescent varieties, attention has been given only to certain yellow and blue chromogenic forms.

A further illustration of the scant attention given to them can be obtained from the work of Houston[4] and that of Chester[5] both referred to in the preceding bulletins of this series. Houston describes only one non-spore-forming organism that is not fluorescent; while

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[2] Conn, H. J. Methods best adapted to the study of the soil flora. Id.:18–42. 1917.

[3] Conn, H. J. Spore-forming bacteria in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 58. 1917.

[4] Houston, A. C. Chemical and bacteriological examination of soils. Local Gov't Board, An. Rept. 27 of Medical Off.:251–296. 1898.

[5] Chester, F. D. Study of the predominating bacteria in a soil sample. Del. Agr. Exp. Sta., Rept. 14:52–66. 1903.

Chester, among the three predominating micro-organisms in a certain soil sample, found only the least abundant one of the three to be without spores. In Chester's case, as already mentioned (Tech. Bul. 57, p. 33), this might have been due to a short incubation period. Houston's results might have been due in part to a short incubation; but as he mixed his soil with sterile water and allowed the mixture to stand an hour before plating, he furnished a good opportunity for those types to predominate (*Ps. fluorescens*, for example) that grow well in water.

In other words, the large group of non-chromogenic, asporogenous bacteria that produce punctiform colonies on agar or gelatin has been given very little attention, and the non-motile members of this group have been practically overlooked. Considering what a large proportion of the colonies on the plates are of this type, it seems strange at first thought that they have been overlooked; and yet the reason is not far to seek. These bacteria grow poorly in ordinary culture media. In laboratory culture, their activities are so insignificant that they can scarcely be measured by chemical tests. It has been assumed that organisms so inactive in the laboratory must also be inactive in soil.

CLASSIFICATION.

THE TWO PRINCIPAL GROUPS.

Two main groups of non-spore-formers have been recognized in this work. The first is the group that liquefies gelatin rapidly, the second the group that produces mere punctiform colonies in gelatin. The latter group contains some slow liquefiers and some non-liquefiers. At first thought it would seem as tho a much more natural division to make would be to distinguish liquefiers from non-liquefiers, but that has not proved to be the case. The bacteria found in the soil which liquefy gelatin slowly seem to be more nearly related to those which do not liquefy it at all than they do to those which liquefy rapidly.

This division has also proved easier to recognize in practice than it would have been to draw the line between liquefiers and non-liquefiers. Many slow-liquefiers do not begin to liquefy at the end of seven days' incubation, and cannot be distinguished from non-liquefiers by means of their colonies. The rapid-liquefying group, however, can always be distinguished by the fact that its colonies are over 1 cm. in diameter, and if given time often liquefy the entire plate; while the colonies of the other group never become as large as 1 cm. in diameter, however long the incubation.

The most important member of the rapidly liquefying group is *Ps. fluorescens* (Flügge) Migula. For convenience sake, therefore, the group may be spoken of as the *Ps. fluorescens* group; but this expression must not be taken to mean that all the organisms are fluorescent. Many of them, indeed, never produce fluorescence, and non-fluorescent strains of *Ps. fluorescens* itself are often encountered. The rapid liquefaction of gelatin is the most striking common characteristic of this group. No non-liquefying fluorescent organisms have been encountered.

The other group of asporogenous bacteria here discussed probably does not contain any well-known organism. They grow so poorly in liquid media that all efforts to characterize species within the group have so far proved fruitless. They have been spoken of in previous publications [6] as the group of "slow-growers" because of their tiny colonies and their poor growth in laboratory media. This term, however, gives a false conception of their importance in soil; for, in soil, conditions probably favor their growth and there is reason to believe that they multiply rapidly there.

In Cornell Bulletin No. 338 the non-spore-formers were classified according to the tests called for by the classification card of the Society of American Bacteriologists.[7] Later work has indicated that except in the case of *Ps. fluorescens*, these tests are of little value. In the present bulletin, therefore, the slowly liquefying asporogenous organisms are merely classified into a few general groups.

RAPID-LIQUEFIERS.

Ps. fluorescens (Flügge) Migula. The most striking characteristic of this type is its fluorescence, which is observed in broth, beef-extract-peptone agar, and sometimes in gelatin. Ability to produce fluorescence is often lost, however, and then the type must be recognized by its other characteristics, such as: rapid liquefaction of gelatin, uniform turbidity in broth, cloudy, structureless colony in gelatin, and acid production from dextrose.

Morphology: Rods 0.4 to 0.8 by 0.8 to 1.5 microns, in old cultures nearly the same shape and size as in young cultures. Flagella 3 to 6, arranged in a clump at one pole. Motility great. Rods do not form chains.

Cultural characteristics: Good growth in broth; no surface growth, uniform turbidity, causing distinct cloudiness of medium; sediment scant or none. Gelatin colonies liquefying with great rapidity; round to irregular in shape, cloudy, structureless, occasionally fluorescent. Growth on agar streak cultures, smooth, soft, glistening, generally causing the medium to show a green fluorescence.

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[6] See especially: Conn, H. J. A classification of the bacteria in two soil plats of unequal productivity. Cornell Agr. Exp. Sta. Bul. 338:65–113. 1913.

[7] For a discussion of this card see Technical Bulletin No. 57, pp. 33 to 40.

Physiology: The typical group number[8] is: Ps. 211.2332133. Fairly consistent results can be obtained in determining its group number, altho certain variations occur. Occasionally there is no acid from dextrose. Some of the cultures reduce nitrates. The power of producing fluorescence is often lost. These variations may indicate the existence of separate species that are now grouped under this one head. In Cornell Bulletin No. 338, several types (Nos. 10,

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[8] The group number, as used on the classification card of the Society of American Bacteriologists, is as follows:

100.	Endospores produced
200.	Endospores not produced
10.	Aerobic (strict)
20.	Facultative anaerobic
30.	Anaerobic (strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.04	No growth with lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.004	No growth with saccharose
.0001	Nitrates reduced with evolution of gas
.0002	Nitrates not reduced
.0003	Nitrates reduced without gas formation
.00001	Fluorescent
.00002	Violet chromogens
.00003	Blue “
.00004	Green “
.00005	Yellow “
.00006	Orange “
.00007	Red “
.00008	Brown “
.00009	Pink “
.00000	Non-chromogenic
.000001	Diastasic action on potato starch, strong
.000002	Diastasic action on potato starch, feeble
.000003	Diastasic action on potato starch, absent
.0000001	Acid and gas from glycerin
.0000002	Acid without gas from glycerin
.0000003	No acid from glycerin
.0000004	No growth with glycerin

The genus according to the system of Migula is given its proper symbol which precedes the number thus:

Bacillus coli (Esch.) Mig. becomes B. 222.111102

For a more complete discussion of the group number see Technical Bulletin No. 57, pp. 33–40. (This report, pages 117–126.)

11, 12, 17, 18 and 19) were recognized on the basis of these variations in physiology. Now that the tests have been found to give inconsistent results, it seems wisest for the present to consider all the cultures as belonging to one type.

Orange, liquefying type.— This has not yet been identified with any previously described species, and its characteristics have not yet been determined definitely enough to justify a new name. It is characterized by its very slender rods, its orange colony in gelatin, and the difficulty with which it can be kept under cultivation in the laboratory.

Morphology: Rods 0.2 to 0.4 by 0.8 to 2 microns. The diameter of the rods seems to be fairly constant in old and in young cultures; but there is apparently a tendency for long rods to occur in young cultures, short rods in old cultures. A single polar flagellum has been observed, but its demonstration is difficult. Motility slight. Rods often grow in long chains.

Cultural characteristics: Growth in broth poor or none. Gelatin colonies on plates made directly from soil, 1 to 2 cm. in diameter, liquefied, orange, containing granules that tend to be radiately arranged; after cultivation in laboratory, cultures produce much smaller and less characteristic colonies that resemble the original colonies only in color. Growth on agar streak cultures, smooth, soft, glistening, bright yellow to orange.

Physiology: Cultures grow poorly in the laboratory, and generally die out after a few transfers. Consequently study is difficult. Variation in physiological reactions is so great that no group number can be assigned. The first three figures are 211; but the rest, with the exception of that referring to chromogenesis, are variable. The cause of the variation is undoubtedly the poor growth in laboratory media.

But for its strikingly slender rods and its orange chromogenesis, it would be doubtful whether cultures showing such variations in physiological reactions should be considered to form a distinct type. These two features, however, are distinct enough so that the type was recognized even when Cornell Bulletin 338 was written. In that bulletin it was denoted as type No. 16.

Other types.— Besides these two fairly distinct types, a number of other rapidly liquefying non-spore-formers have been found. No study of them has been made as yet. They are generally present in soil, altho not in large numbers. They have been observed in large numbers in manured soil, together with the two above types.

None of these organisms have been identified with previously described species; but they seem to be closely related to *Ps. fluorescens.* They are small, short rods, generally with polar flagella. Their colonies in gelatin are non-characteristic — about 0.5 to 1 cm. in diameter, usually with the center gray and denser than the surrounding clear zone of liquefied gelatin. Sometimes it is almost impossible to distinguish their gelatin colonies from atypical *B. megatherium* colonies.

"SLOW-GROWERS."

GENERAL CHARACTERISTICS.

This group probably does not contain any well-known organism. It is a group of small, short rods or cocci, rarely over one micron in length or over half a micron in diameter, characterized by poor growth in all liquid media, and by the production of punctiform colonies in gelatin. On agar streak culture they grow fairly well, producing a soft, smooth, glistening, slimy to watery growth.

The actual number of different kinds of bacteria in this group is unknown. There are very few morphological differences upon which to base distinctions; and because of their poor growth in liquid media, it is not possible to use the ordinary physiological tests to effect a classification. Some new system of classification must be applied to these organisms, but no satisfactory system has yet suggested itself. The only classification attempted at present is to arrange them in five groups on the basis of three characteristics: shape, chromogenesis, and liquefaction of gelatin.[9]

I. ROD-SHAPED FORMS.

A. Chromogenic.— The rods are 0.1 to 0.7 by 0.4–1.5 microns. They do not form chains. Motility has not been observed. Cultural characteristics are not distinctive, except for the lemon-yellow chromogenesis on beef-extract-peptone agar. The typical group number is Bact. 211.33335?3, altho there is some variation in regard to nitrate reduction and gelatin liquefaction, two cultures not reducing nitrates, and three not liquefying gelatin.

In Cornell Bulletin No. 338, two types, Nos. 22 and 29 were separated on the basis of nitrate reduction. Both are now grouped together under this one head.

This yellow-chromogenic type is not so abundant in the soils studied as the non-chromogenic ones, only 23 of the 330 cultures of "slow-growers" proving to be of this type.

B. Non-chromogenic: 1. Liquefying gelatin slowly.— Rods vary in size from 0.2 by 0.5 to 0.8 by 1.5 microns. They do not form chains.

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[9] As already stated (Tech. Bul. 58, p. 13), certain cultures isolated from punctiform colonies have proved to be spore-producers. It is difficult to know where to place them in the present classification. They plainly do not belong with the rest of the "slow-growers," and yet they do not seem to be closely related to other spore-formers. These peculiar bacteria, however, have never been found on gelatin plates from soil, unless the soil infusion was heated (75–85° C.) before plating. The present investigation is limited to those organisms growing on gelatin plates made in the ordinary way; so these slow-growing spore-formers have not been studied. Their presence in soil must be mentioned, however, and they should not be omitted from a complete flora study of soil.

Motility has rarely been observed, but sometimes a tuft of polar flagella has been demonstrated. This may mean that there are really two types, one motile, the other immotile; but because of the difficulty in determining motility, this distinction is not recognized here. It is possible that all the cultures might prove to be motile if good methods of determining their motility were known.

Cultural characteristics are not distinctive. The typical group number is Bact. (or Ps.?) 211.33320?3, with some variation in regard to the production of acid from dextrose and nitrite from nitrate, an occasional culture having acidified dextrose and reduced nitrate.

Types 20, 21, 25, 26, 27 and 28, as described in Cornell Bulletin No. 338, should be included under this head.

This is the most common type of "slow-growers" in the soils studied, as 180 out of the 330 cultures proved to be of this type.

2. Not liquefying gelatin.— The rods are 0.3 to 0.8 by 0.6 to 1.5 microns. They do not form chains. Motility is often observed. Flagella, if present, have always been found to be polar. Cultural characteristics are not distinctive. The typical group number is Ps. 212.33320?3, a few cultures differing from this in regard to the reduction of nitrate or the acid production from dextrose.

Types 31 to 34, 36 and 37 of Cornell Bulletin 338, are all included under this head.

Of the 330 cultures of "slow-growers" studied, 95 were of this type. It proved about three times as abundant in the soils studied at Geneva as in the soil studied at Ithaca.

II. COCCI.

A. *Liquefying gelatin slowly.*— The typical group number is M. 211.33320?3.

B. *Not liquefying gelatin.*— The typical group number is M. 212.33320?3.

These two types are much alike, and perhaps should not be separated, inconsistent results having been obtained in testing for liquefaction of gelatin.

These organisms show very little variation in size or shape, the diameter of the cocci varying only from 0.5 to 0.8 microns. Their cultural characteristics are not distinctive. In physiological reactions the two group numbers given above were obtained fairly consistently, except that a few cultures acidified dextrose broth or reduced nitrates.

Only one type of cocci was mentioned in Cornell Bulletin 338. This was a non-liquefying type and was denoted as No. 38.

Only 26 cultures of cocci have been found thruout the work. They are not regarded as characteristic of soil. Past writers have occasionally described cocci as present in soil; but they have never been stated to be abundant there.

RECOGNITION OF COLONIES ON PLATES.

Unfortunately the non-spore-forming bacteria in general produce colonies on gelatin and agar that do not have striking characteristics. *Ps. fluorescens* and the orange, liquefying type are the only exceptions to this statement. *Ps. fluorescens* can be recognized on gelatin by its large, rapidly liquefying colony, with entire margin, faintly cloudy but almost structureless, which will liquefy an entire plate if given time; but its agar colonies are less characteristic, fluorescence generally occurring on beef-extract-peptone agar, but not constantly enough to characterize the species. The orange, liquefying type can be recognized by its fairly large orange colonies; on gelatin they are sometimes as large as 2 cm. in diameter and show a striking radiate structure.

The other non-spore-forming bacteria all produce non-characteristic colonies. The punctiform colonies can be distinguished from the rapidly liquefying ones; but that is all the classification that can be made. This is unfortunate, because it is impossible to tell how numerous any type may be in a particular soil sample unless its colonies on the plates can be recognized. For this reason the only information at hand as to the relative abundance of the different types of " slow-growers " just mentioned has been obtained from the number of cultures of each found in a series of chance isolations made from a large number of punctiform colonies.

About 1 to 3 per ct. of the colonies on gelatin are of a type which may be produced by certain spore-formers and certain non-spore-formers alike. This has caused some difficulty in computing the total number of non-spore-formers present in a given sample. There is evidence, however, which indicates that about three-quarters of these colonies are non-spore-formers; and in obtaining the figures given in Tables I to III, an estimate has been made upon that basis. Because of the small number of these colonies ordinarily observed, the possible error thus introduced is slight.

The only way to obtain more accurate data than this as to the abundance of the different non-spore-forming bacteria in soil is to devise some medium upon which they all produce characteristic colonies. No agar or gelatin medium yet investigated is satisfactory for this purpose. Agar is likely to prove more satisfactory in this respect than gelatin; for the simpler chemical composition of agar itself makes it easier to control the composition of an agar medium than of a gelatin medium. One of the greatest needs at present for the successful study of soil bacteria is a medium upon which the colonies of the asporogenous types can be recognized.

DISTRIBUTION.

ABUNDANCE IN SOIL.

The great abundance of non-spore-forming bacteria in soil can be seen by a glance at Table I, in which are listed a series of gelatin plate counts [10] made from a single soil plat at intervals thruout the course of three years. In the twenty-seven analyses listed in this

TABLE I.—GELATIN PLATE COUNTS OF FIELD SOIL.

DATE.	Moisture content.	NUMBER OF COLONIES PER GRAM OF DRY SOIL.		Remarks.
		Non-spore-forming bacteria.	Other colonies (spore-formers and Actinomycetes).	
	Per ct.			
Nov. 24, 1911	25.	22,500,000	5,500,000	
Jan. 13, 1912	40.	28,500,000	7,500,000	Frozen 8 days.
Jan. 23, 1912	37.	31,000,000	9,000,000	Frozen 18 days.
Feb. 13, 1912	43.	11,000,000	10,000,000	Frozen 39 days.
Mar. 1, 1912	37.	20,500,000	6,500,000	Frozen 57 days.
April 24, 1912	23.4	18,000,000	4,500,000	
May 6, 1912	40.	20,000,000	9,000,000	
June 5, 1912	20.4	19,200,000	4,500,000	
Sept. 23, 1912	22.5	28,500,000	10,500,000	
Oct. 25, 1912	24.5	14,500,000	5,500,000	
Dec. 3, 1912	26.2	28,500,000	6,500,000	
Jan. 15, 1913	39.5	14,000,000	6,000,000	Frozen 7 days.
Feb. 5, 1913	22.	22,300,000	7,700,000	Frozen 4 days.
Feb. 14, 1913	26.2	44,000,000	10,000,000	Frozen 13 days.
Mar. 11, 1913	38.8	22,000,000	7,000,000	Partly frozen.
April 4, 1913	22.8	19,300,000	7,700,000	
July 10, 1913	17.	16,800,000	5,200,000	
Nov. 26, 1913	21.5	11,800,000	4,200,000	
Dec. 15, 1913	19.6	7,800,000	3,200,000	
Jan. 16, 1914	31.2	15,500,000	3,500,000	Frozen 9 days.
Jan. 30, 1914	24.6	26,000,000	7,000,000	Thawed 1 day.
Feb. 7, 1914	27.7	22,700,000	7,300,000	Partly frozen.
Feb. 26, 1914	32.	31,000,000	7,000,000	Frozen 21 days.
April 15, 1914	20.	13,700,000	7,300,000	
April 29, 1914	20.	11,800,000	4,200,000	
Aug. 7, 1914	9.	5,000,000	4,000,000	
Aug. 19, 1914	20.	18,000,000	4,700,000	

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[10] The figures in this table and in the others that follow are always the average of at least three individual plate counts. Two dilutions were always used, and three or four gelatin plates made of each dilution. When the figures obtained from one dilution checked with those of the other, the average included all of the individual plate counts; when they did not check, it included only those of that dilution having

table, the non-spore-formers have averaged 75.6 per ct. of all the colonies on the plates. This has been found to be about the general average in cultivated soil; in sod soil (in which, as already stated,[11] the number of Actinomycetes is higher than in cultivated soil) the percentage of non-spore-formers is somewhat lower.

The majority of this 75 per ct. is composed of the " slow-growers." The rapid-liquefying group is not a very abundant one in the soils studied. In many cases it is either lacking or not abundant enough to appear on plates in the dilutions used; and the largest number ever found, except in manured soil, is about 1,500,000 per gram of soil or about 5 per ct. of all the colonies developing on the plates. The group of " slow-growers," on the other hand, is very abundant. Excluding air-dried soil, where their numbers are quite low, and manured soil, where their numbers are extremely high, plate counts have ranged from about 2,000,000 per gram to about 60,000,000 per gram.

FLUCTUATIONS IN NUMBERS.

The mere fact that the majority of the bacteria developing on plates made from soil are non-spore-formers suggests that these organisms are important in soil; but still stronger evidence of their importance comes from the fact that they fluctuate greatly in numbers. In this respect they differ greatly from the spore-forming bacteria, which as already mentioned [12] are relatively constant in numbers. The figures in Table I illustrate the fluctuations in their numbers. During the three years over which the sampling was continued the plate count of non-spore-formers has varied from 5,000,000 to 44,000,000 per gram; while all the other organisms growing on the plates (spore-forming bacteria and Actinomycetes) have varied only from 3,200,000 to 10,500,000 per gram.

A further illustration of this same fact can be obtained from the data already published in Cornell Bulletin 338. It will be seen from Table II in that bulletin (p. 87) that the number of colonies of "slow-growers" varied from 2,000,000 to 25,000,000 per gram; while (excluding the first two counts in the table, which as stated in that bulletin are probably inaccurate) the number of all the other colonies varied only from 1,700,000 to 8,800,000 per gram.

Even more striking fluctuations in the number of non-spore-formers have been observed in pot experiments. The aeration brought about by potting soil is generally admitted to cause an

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between 50 and 100 colonies per plate (or nearest to this number, in case neither dilution gave plates within these limits). In almost all cases, moreover, parallel plates were made with some agar medium; and altho the counts given in the tables were never obtained from the agar plates, the use of agar sometimes helped to show which of two disagreeing gelatin plate counts was correct.

[11] Conn, H. J. A possible function of Actinomycetes in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 52. 1916. *Jour. Bact.*, 1:197–207. 1916.

[12] See footnote 3.

increase in the numbers of bacteria. In the course of the present work it has been noticed that the numbers of spore-formers and Actinomycetes remain about constant after aeration and that only the non-spore-forming bacteria increase noticeably in numbers. A good illustration of this is given in Table II. The soil used in this experiment was kept in a pot in the laboratory, watered occasionally so as to keep the moisture content at about 10 to 20 per ct. The soil placed in this pot was obtained from a field plat from which plates had been made at intervals for two or three years, so that the composition of its flora was fairly well known. The first count listed in the table is a typical analysis of this soil before aeration. It will be seen that during the first week or two after aeration the count of rapid-liquefying non-spore-formers rose to about four times its normal height, remaining high during the first month, afterward returning to normal height or even lower. The number of punctiform colonies increased immediately to twice normal, and did not return to its original level until after the first month, and then only temporarily, later on increasing again to more than twice its normal height. Meanwhile the number of colonies of other organisms (Actinomycetes and spore-formers) remained strikingly constant, the lowest count being 2,700,000 and the highest 4,700,000 per gram, and such fluctuations as they did show in their numbers bore no relation to the aeration.

TABLE II.— GELATIN PLATE COUNTS OF AERATED SOIL.

Figures indicate number of colonies per gram of dry soil.

TIME SINCE AERATION	NON-SPORE-FORMING BACTERIA		OTHER COLONIES: (Spore-formers and Actinomycetes)
	Rapid-liquefying colonies	Punctiform colonies	
(Unaerated)	350,000	11,000,000	4,700,000
1 day	1,500,000	22,500,000	4,000,000
5 days	1,250,000	18,000,000	3,000,000
13 days	1,500,000	24,000,000	4,500,000
22 days	1,000,000	16,000,000	3,000,000
3 months	300,000	10,000,000	3,200,000
3 months, 25 days	700,000	27,500,000	4,500,000
4 months, 8 days	300,000	16,000,000	2,700,000
4 months, 10 days	600,000	23,000,000	3,400,000
4 months, 15 days	350,000	19,000,000	4,700,000
7 months, 15 days	150,000	12,000,000	4,000,000
10 months	100,000	4,800,000	3,000,000

Other experiments have been performed which illustrate the fact that following aeration of the soil or the addition of organic matter, the greatest numerical increases occur in the group of non-spore-forming bacteria. There is no need of giving all the data, but the experiment summarized in Table III is striking enough to deserve mention. In this case two pots were filled with the same soil, fresh horse manure having been added to one but not to the other. The pots were kept in the laboratory and watered as in the experiment just described. In the manured soil the number of rapidly liquefying colonies of non-spore-formers and the number of punctiform colonies each increased to about one hundred times normal during the first two weeks after manuring, and then gradually subsided, reaching their normal level after six months. Meanwhile the number of colonies of other kinds merely increased to about twice normal; which slight increase might easily have been due to the inaccuracy introduced by the high dilutions necessary. A comparison between manured and unmanured pots, shows plainly that the immense difference between the total counts of these soils was due wholly to differences in numbers of the non-spore-formers.

TABLE III.— GELATIN PLATE COUNTS OF AERATED SOIL, MANURED AND UNMANURED.

Figures indicate number of colonies per gram of dry soil.

TIME SINCE ADDING MANURE TO MANURED SOIL.	UNMANURED SOIL.			MANURED SOIL.		
	NON-SPORE-FORMING BACTERIA.		OTHER COLONIES: (Spore-formers and Actinomycetes.)	NON-SPORE-FORMING BACTERIA.		OTHER COLONIES: (Spore-formers and Actinomycetes.)
	Rapid-liquefying colonies.	Punctiform colonies.		Rapid liquefying colonies.	Punctiform colonies.	
(Original soil)*...	100,000	3,400,000	6,500,000	100,000	3,400,000	6,500,000
1 day.........	....	....	....	4,700,000	190,000,000	5,000,000
3 days.........	....	....	....	25,000,000	370,000,000	5,000,000
6 days.........	....	....	....	28,000,000	280,000,000	8,000,000
9 days.........	....	....	....	29,000,000	125,000,000	6,000,000
15 days.........	....	....	....	38,000,000	150,000,000	7,000,000
19 days.........	325,000	5,000,000	5,700,000	10,000,000	48,000,000	8,000,000
1 month.......	....	....	....	4,500,000	53,000,000	12,500,000
1 month, 15 days	75,000	4,000,000	5,500,000	2,000,000	32,000,000	10,000,000
2 months.......	300,000	4,500,000	4,500,000	1,300,000	27,000,000	8,700,000
4 months.......	350,000	9,000,000	5,700,000	1,500,000	15,000,000	5,500,000
6 months.......	200,000	12,000,000	4,800,000	150,000	7,000,000	3,850,000
8 months.......	75,000	3,500,000	5,500,000	450,000	8,500,000	6,500,000
13 months.......	100,000	1,700,000	2,700,000	200,000	4,200,000	3,600,000
15 months.......	75,000	3,000,000	2,500,000	....	....	....

* The count obtained from the original soil is listed under the heading " Manured Soil " as well as under " Unmanured Soil," because this same soil was used in both the manured and unmanured pots.

These data in regard to manured soil are surprising as well as interesting. The spore-forming bacteria and the Actinomycetes have frequently been mentioned as probably taking part in the decomposition of manure; but in this experiment they remained almost constant in numbers while decomposition of the manure was going on rapidly — as proved by the strong odor of ammonia, if by nothing else. This experiment was originally planned with the idea of furnishing favorable conditions for the growth of spore-formers in soil; and it was with some surprise that they were observed to remain constant while the non-spore-formers increased in numbers enormously. This experiment has not yet been repeated; so the conclusion that spore-formers and Actinomycetes do not take part in the decomposition of manure in soil is not yet justified. The findings suggest that decomposition of manure is brought about by the non-spore-forming bacteria; but they are summarized here merely as an illustration of the fact that they are the organisms which show the greatest fluctuations in number whenever the total numbers of bacteria increase or decrease.[13] Further interesting information as to the occurrence of *Ps. fluorescens* was obtained from an experiment planned for an entirely different purpose. Some quartz sand was mixed with nutrient material, placed in tumblers and sterilized. After sterilization the tumblers were inoculated in various ways, but in no case with *Ps. fluorescens.* No attempt was made to prevent contamination with air-borne organisms, as it was desired to see which of the organisms were capable of prevailing over rival organisms under the conditions of the experiment. Fifteen out of 16 tumblers were later found to be contaminated with *Ps. fluorescens,* which developed to considerable numbers and in some cases persisted for a year. Plainly this organism found conditions in these tumblers well suited to its growth. The exact meaning of this fact is still unexplained; but these results, taken in connection with those given in Tables II and III, suggest that *Ps. fluorescens* is pre-eminently suited to growth in moist and well aerated soil or sand.

SIGNIFICANCE OF THE NON-SPORE-FORMING BACTERIA IN SOIL.

It has been emphasized in the past that the most abundant bacteria in soil are not necessarily the most important ones; so the large numbers of non-spore-formers found in soil must not be accepted alone as proof of their importance. The evidence generally considered necessary to corroborate that obtained from numbers alone is to show that they can carry on some activity known to take place in the soil; but evidence of this sort in regard to the asporogenous bacteria has been obtained only in the case of the group of rapid liquefiers. *Ps. fluorescens* is known to be an ammonifier; and all the members of the group have proteolytic powers. This, together with the fact that they have been found to be especially abundant in freshly manured soil, suggests that they may be the important soil ammonifiers.

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[13] A further illustration may be obtained from the data to be published in Technical Bulletin 60 (Actinomycetes in soil) Table II. In this case a large increase in the non-spore-forming bacteria was noticed after mixing grass roots with soil.

The activities of the " slow-growers " are more problematic. Some do not liquefy gelatin, while others liquefy it so slowly that the importance of their proteolytic ability is at least questionable. Nevertheless, the increase in their numbers in the soil to which manure was added (see Table III, p. 153) suggests that in this experiment, at least, they took part in some stage of the decomposition of manure. Winogradsky's nitrifiers are non-spore-forming bacteria, and the idea has suggested itself that nitrification might be the function of some of the asporogenous organisms studied in the present work. It is not likely, to be sure, that any of the forms which grow on gelatin plates are nitrifiers, because Winogradsky's nitrifiers did not grow on gelatin; but the possibility is not entirely ruled out of court, for in the present work the gelatin has been of a different composition and the general technic quite different from that used by Winogradsky and others who have studied the nitrifiers. For this reason about ten cultures, isolated from punctiform colonies, were inoculated into a mixture of sand and Omelianski's solution,[14] but no signs of nitrite appeared. No definite statement, of course, can be made on the basis of such meager data, and when a more complete study of these organisms is made, nitrifying power is one of the tests that must be included. For the present their function in the soil remains a matter of speculation. It is hoped that this paper will interest others in them, so that they may be given a more thoro study in the future.

A stronger assertion of the importance of asporogenous bacteria in soil could be made if their functions were known; but one fact brought out by the present investigation is fully as good an argument for their importance as a knowledge of their activities in pure culture would be. This fact is that when any change in soil conditions causes an increase in the number of bacteria present, the greatest increase always seems to occur in the group of non-spore-forming bacteria. This plainly indicates that they are active in soil; and considering that they comprise over half of the soil flora, it is hard not to believe that they are important.

The knowledge that they are active in soil is perhaps a better argument for their importance than would be a knowledge of their activities in laboratory culture. Knowledge of the activities of an organism in pure culture may lead to a very false conception of its function in soil; as seems to have been the case when it was assumed that the members of the *B. subtilis* group were the important soil ammonifiers. As the asporogenous bacteria have been shown to be active in soil, no misconception of this sort seems possible in regard to them; altho, while their activities still remain unknown, the question is naturally raised as to what may be their significance. This question must be the subject of future investigation.

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[14] Omelianski, V. Ueber die Isolierung der Nitrifikationsmikroben aus dem Erdboden. *Centbl. Bakt.*, II Abt., **5**:537–549. 1899.

SOIL FLORA STUDIES.*

V. ACTINOMYCETES IN SOIL.

H. JOEL CONN.

SUMMARY.

1. Ordinarily from twelve to fifty per ct. of the colonies on plate cultures made from soil are those of Actinomycetes.

2. Description of species of Actinomycetes is at present very difficult. The literature abounds with descriptions that have become invalidated as better methods of study have been developed.

3. About seventy different types have been found in the soils studied. Three of them are of fairly common occurrence. One of these three, ***A. pheochromogenus,*** n. sp., is considered distinct enough to be given a specific name. One of the other two types agrees in cultural characteristics with the potato-scab organism, but its pathogenicity has not yet been tested. The other common type probably is not a distinct species. Classification of the less common types is left for a more complete publication on these organisms which, it is hoped, will appear later.

4. The significance of these organisms in soil needs further investigation. There are good indications that they are active as well as numerically important.

INTRODUCTION.

Of the previous papers of the present series of Soil Flora Studies, the first two[1] were introductory, and the next two[2] each considered one of the large groups of soil micro-organisms. Both of these two groups were groups of true bacteria. The present paper, however, takes up the third large group of soil micro-organisms, the Actinomycetes, which are generally spoken of as higher bacteria, but are sometimes considered to belong with the true fungi rather than with the bacteria.

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[1] Conn, H. J. The general characteristics of the microscopic flora of soil. N. Y. Agr. Exp. Sta., Tech. Bul. 57: 1–17. 1917. Methods best adapted to the study of the soil flora. Id.:18–42. 1917.

[2] Conn, H. J. Spore-forming bacteria in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 58. 1917. Non-spore-forming bacteria in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 59. 1917.

* Reprint of Technical Bulletin No. 60, March, 1917.

The present paper, like the preceding one (on non-spore-forming bacteria), is merely preliminary. It has not yet proved possible to distinguish species among the Actinomycetes or among the non-spore-forming bacteria with as much success as among the spore-forming bacteria. While three or four common species of spore-forming bacteria have been recognized with some degree of certainty, it has proved impossible to establish beyond question the identity of more than one species of Actinomycetes or of non-spore-forming bacteria. In regard to their activities, moreover, only enough has been learned to indicate that they are important in soil, but not enough to show what their importance may be.

There is great need, therefore, of more work on the Actinomycetes and non-spore-forming bacteria of soil. At the present time especial attention is being given at this Station to the Actinomycetes. A study is being made of their morphology, of their physiological activities in soil and in culture media, and of the constancy of the various characteristics that may be used for the separation of species. The present paper is merely a report of progress on this study. A more complete publication on the subject may be expected later.

GENERAL DESCRIPTION OF ACTINOMYCETES.

The genus *Actinomyces* Harz, em. Gasperini, is characterized by the possession of a mycelium composed of hyphæ which show true branching, like that of the higher fungi. These hyphae are very delicate, however, in comparison with the hyphæ of higher fungi, (seldom measuring over 2 microns in diameter) and to judge by their staining reactions, resemble true bacteria in their protoplasmic properties. Their growth is not wholly within the agar or gelatin medium upon which they have been inoculated; for when conditions favor, an aerial mycelium is produced. In the aerial mycelium " conidia " are formed. These conidia are sometimes round, sometimes oval, and sometimes rod-shaped. They resemble bacteria closely in size, shape, and staining properties. They are generally between 0.6 and 1.5 microns in diameter, and if oval or rod-shaped, between 1 and 2 microns long. They stain readily with ordinary bacterial stains, and in a microscopic preparation which does not contain any hyphæ, often cannot be distinguished from true bacteria. In many cases deep-stained granules show at the poles, strongly suggestive of the metachromatic granules of the diphtheria organism. According to Sanfelice,[3] some of the Actinomycetes are acid-fast like the tubercle organism. The diphtheria and tubercle organisms, moreover, sometimes produce branching forms, and some writers place these two organisms in the same group with *Actinomyces*.

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[3] Sanfelice, F. Ueber die pathogene Wirkung einige Streptothrix- (Actinomyces-) Arten. *Centbl. Bakt.*, I Abt., Orig., **36**:355–367. 1904.

The growth of Actinomycetes on solid media is very characteristic. The mass of growth is generally of a tough, leathery consistency, sometimes smooth, sometimes wrinkled, and often piled high above the surface of the medium. Often the mass is brilliantly colored, and the color produced varies greatly with differences in the composition of the medium, but with constant composition of the medium, the color of the growth may be characteristic of the species. The aerial mycelium, often produced above this growth, may also be brilliantly colored and of an entirely different color from the mass of growth beneath it. Sometimes the aerial hyphæ are short and give the growth a chalky or mildewy appearance; but often they are long enough to cover the growth with a light, delicate nap, one or two millimeters thick. Some species produce pigments that diffuse thru the medium, coloring it gray, yellow, brown, red, blue or green. The color varies with the species and with the composition of the medium. It is not so definitely characteristic of the species as is the color of the growth itself or of the aerial mycelium; but with a medium of constant composition, the color produced is of considerable value in the recognition of species. On gelatin there is less diversity of growth than on agar. The growth is generally gray, brown or colorless; the aerial mycelium is often lacking, and if present is white, gray or colorless; and if the medium itself is colored, it generally becomes a reddish brown.

The growth in liquid media is also characteristic. The medium remains clear except for small colonies that may sink to the bottom, remain in suspension, float on the surface, or adhere to the walls of the tube. The surface colonies often grow together and become covered with a mass of aerial mycelium, sometimes forming a firm, wrinkled membrane that strongly suggests the surface membrane of the tubercle organism growing on broth. Pigments are often produced in liquid culture, the pigment varying with the composition of the medium and with the species growing in it.

The physiology of these organisms has never been thoroly studied. There is no agreement yet as to the media best suited for physiological studies of them, and each investigator has used media of his own. This fact, together with the confusion in nomenclature, has prevented concordant results. Nearly all liquefy gelatin and ammonify proteid, Münter [4] claiming that ammonification is their chief function. Nitrate-reduction has often been observed, as has the decomposition of cellulose. Some are animal pathogens and at least one a plant pathogen. Other important physiological activities will undoubtedly be worked out when the technic for studying them is further developed. It is not impossible that they are as diverse in physiology as are the true bacteria.

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[4] Münter, F. Ueber Stickstoffumsetzungen einiger Aktinomyceten. *Centbl. Bakt.*, II Abt. 39:561–583. 1914.

One of the most characteristic features of many members of this group is their peculiar odor. It is a pungent, musty odor, difficult to describe, but impossible to mistake after once having it brought to the attention. It is sometimes spoken of as an earthy odor; but it would be more correct to say that soil often has an Actinomyces-odor, as the odor of the cultures is much stronger than that of soil, and the soil odor is undoubtedly due to the Actinomycetes it contains. The odor seems to be associated with the aerial conidia, and does not seem to be produced by cultures that do not possess aerial mycelium. Not all species of *Actinomyces* have this odor, however, even when an abundant aerial mycelium is produced.

HISTORICAL.

THE QUESTION OF NOMENCLATURE.

A full account of previous work will have to be omitted in this preliminary paper. In Krainsky's recent paper[5] there is a very complete discussion of literature, which is easily available to anyone wishing a more complete review of the subject. For the present a brief resumé of the work bearing on nomenclature and classification must suffice, leaving a full review for the later paper.

The first member of this group to which a specific name was given was *Streptothrix Foesteri* Cohn 1875.[6] This species was not described definitely enough for identification, a fact which undoubtedly explains why some have claimed it to be pathogenic, others non-pathogenic. The species *Actinomyces bovis* Harz 1877, on the other hand, has been definitely associated with bovine actinomycosis (" lumpy jaw ") and is generally regarded as a distinct species. Even in regard to *A. bovis,* however, there is some dispute, some having claimed it to be aerobic and others to be anaerobic.

Great confusion has arisen as to the phylogenetic relationships of these organisms, Migula[7] having placed them as bacteria in the genus *Cladothrix*, while Savageau and Radais[8] placed them with *Hyphomycetes* in the genus *Oospora*. The opinion of Lehmann and Neumann[9] in this matter stands midway between these two views. They consider the group to stand between the *Schizomycetes* and *Hyphomycetes*, resembling the former in the size of its cells and staining properties of its protoplasm, but resembling the latter in forming branched hyphæ. They place three genera in the group *Actinomycetes: Corynebacterium*, containing the diphtheria organism, *Mycobacterium*, containing the tubercle organism, and *Actinomyces*, containing the forms with true branched mycelium. Chester[10] also places *Actinomyces* (under the name *Streptothrix*) with the tubercle and diphtheria organisms; but considers them as constituting the family *Mycobacteriaceæ* which he places with the true bacteria. This family, according to Chester, contains the two genera *Streptothrix* and *Mycobacterium*, the latter genus containing the tubercle and diphtheria organisms together with the organism of legume nodules. In the present work the genus *Actinomyces* alone is considered, and there is no need of deciding whether it is more closely related to these bacteria or to the simpler *Hyphomycetes* such as *Oospora*. For the sake of clearness, however, it must be stated that when the term Actinomycetes is used in the present bulletin, reference is made to members of the genus *Actinomyces* alone, and not to the whole group *Actinomycetes* as recognized by Lehmann and Neumann; and it must further be stated that the expression " true bacteria " is used to refer to the coccus, rod, and spirillum forms in contradistinction from the filamentous forms, and not to the Eubacteria of Migula, which includes *Actinomyces*.

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[5] Krainsky, A. Die Aktinomyceten und ihre Bedeutung in der Natur. *Centbl. Bakt.*, II Abt., **41**:649–688. 1914.

[6] Cohn, F. Untersuchungen über Bacterien, II. *Beitr. z. Biol. d. Pflanzen.*, **1**:(Hft. 3):141–207. 1875.

[7] Migula, W. System der Bakterien. 1897.

[8] Savageau and Radais. Sur les genres Cladothrix, Streptothrix, Actinomyces. *Ann. Inst. Past.* **6**:242–273. 1892.

[9] Lehmann, K. B., and Neumann, R. O. Atlas und Grundriss der Bakteriologie, Teil II. 1912. See especially p. 158.

Not only is there confusion as to the relationships of these organisms, but there is no general acceptance of the name *Actinomyces* for the genus. Various other names have been used; the names *Streptothrix*, *Nocardia*, *Cladothrix* and *Oospora* are often encountered in the literature today. Of these names, *Streptothorix* is used nearly as often as *Actinomyces*, but is untenable, because it was preëmpted by Corda[11] for a genus of true fungi. *Cladothrix* is applied to a definite genus of higher bacteria (of which *C. dichotoma* Cohn is the type) which is distinctly different from *Actinomyces*. *Oospora* is the name of an ill-defined genus of *Hyphomycetes*, of which the best known member, *O. lactis*, bears little resemblance to *Actinomyces;* and these two genera seem to be distinct, altho some writers have claimed that they should be combined. All these points are thoroly discussed, not only by Lehmann and Neumann[12]; but also by Lachner-Sandoval.[13]

Excluding these names, the only ones admissible are *Actinomyces* Harz (1877) and *Nocardia* Trevisan (1889). Of them, *Actinomyces* must be preferred on grounds of priority, provided only one generic name is used for all these organisms. *Nocardia* may be used for one section of the group when it is subdivided; but attempts to subdivide it have so far proved unsuccessful. Eventually it will probably be subdivided, for the animal pathogens are quite different from the saprophytic forms; but recognition of genera must be preceded by the recognition of species, and no species described in the past can be regarded as characterized sufficiently for recognition except those that are known to be the cause of some definite disease. This makes it difficult at present to obtain any type species for the genus *Nocardia.* Besides animal pathogens, perhaps the only recognizable species is the causal organism of potato scab, which was originally named *Oospora scabies* by Thaxter. It still remains to be shown, however, whether the potato scab organism is sufficiently distinct from animal parasites to be used as the type for a separate genus. For the present, the genus *Actinomyces* Harz, em. Gasperini, is best left undivided.

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[10] Chester, F. D. A manual of determinative bacteriology. 1901.

[11] Corda, A. C. J. Prachtflora europaescher Schimmelbildung, Leipsic and Dresden, 1839.

[12] See footnote 9.

[13] Lachner-Sandoval, V. Ueber Strahlenpilze. Strassburg Univ., Inaug. Dissert. 1898.

EARLY ATTEMPTS AT CLASSIFICATION.

As the first species of *Actinomyces* to attract general attention was a pathogen, the pathogenicity of these organisms has been studied more than their taxonomy. Notwithstanding the fact that the literature is full of names of species, very few of them, even up to the present day, have been described sufficiently for recognition. *A. chromogenus* Gasperini, for example, has been generally regarded as one of the best known saprophytic species, yet, as shown by Krainsky,[14] is a group rather than a species. The most striking feature ascribed to *A. chromogenus* is the production of a brown pigment in gelatin or peptone agar, which rapidly diffuses thru the medium. This characteristic was discussed at some length by Beijerinck [15] who ascribed it to the production of quinon. It is sometimes referred to as "chromogen" production. It proves, however, to be an insufficient basis for the characterization of the species. Krainsky (working in Beijerinck's laboratory) found four types which agreed in every respect with the original description of *A. chromogenus*, and yet were distinct from each other. In the present work at least thirty types have been found, any one of which might be *A. chromogenus*, so far as published descriptions are concerned. Worse yet, the production of this pigment proves not to be a constant characteristic, so that the *A. chromogenus* type cannot even be recognized as a group distinctly marked off from the other types.

In regard to other types, even worse confusion exists. Early investigators cultivated these organisms on the ordinary complex organic media which are of variable composition and are not especially adapted to the growth of these peculiar organisms. On such media,

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[14] See footnote 5.

[15] Beijerinck, M. W. Ueber Chinonbildung durch Streptothrix chromogena und Lebensweise dieses Mikroben. *Centbl. Bakt.*, II Abt., **6**:2–12. 1900.

(as shown by Krainsky) their growth is likely to be variable and non-characteristic; while on synthetic media, less variable and often more characteristic growth may be obtained. This fact prevents the recognition today of the species of Gasperini [16] and of Lachner-Sandoval,[17] who until recently were considered to have done the best systematic work on the group.

The inconstancy of the characteristics of these organisms on ordinary media was recognized by Sanfelice,[18] who abandoned the attempt to recognize species and proposed the establishment of three groups, each centering around a type species just as the *B. coli* group centers around the form described by Escherich. Sanfelice's three groups were: the *A. flavus* group, the *A. albus* group, and the *A. violaceous* group. This contribution of Sanfelice would have been an important step in the progress of classification if he had used media adapted to Actinomycetes, on which their diagnostic characteristics would appear. He apparently did not realize that the use of complex organic compounds, such as peptone, in his media obscured the most characteristic features of the organisms.

KRAINSKY'S WORK.

The important point in Krainsky's contribution [19] to the classification of the Actinomycetes is that he realized that ordinary bacteriological media were unsatisfactory and used media of simple chemical composition. The two media upon which he obtained the best results were a calcium malate agar and a dextrose agar.[20] Basing his classification largely upon the growth on these media, he classified his cultures into eighteen species. He showed that three different kinds of chromogenesis might be used in classification: pigments which color the medium; pigments which color only the colony; and pigments which color the aerial mycelium alone. Each species produces characteristic pigments of each of these three sorts if grown in the right media.

Krainsky's descriptions are undoubtedly the most complete characterizations of saprophytic species of Actinomycetes that have yet been published. Nevertheless his characterizations are not sufficiently complete. Some of the features he has used for distinguishing species are inconstant; and not a sufficient variety of such features were used. It has proved impossible to identify with certainty any organism thus far studied with any species described by Krainsky.

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[16] Gasperini, G. Recerche morfologische e biologiche sul genere Actinomyces-Harz. *Ist. d'Igiene Speri. dell' Univ. Roma.*, N. S. **2**:167–229. 1892.

[17] See footnote 13.

[18] See footnote 3.

[19] See footnote 5.

[20] Krainsky's calcium malate agar contains 10 g. calcium malate, 0.5 g. NH_4Cl, and 0.5 g. K_2HPO_4 to the litre (concentration of the agar not stated). It has been modified in the present work by using 5 g. instead of 10 g. of calcium malate, because it has not been found possible to dissolve 10 g. in one litre of water. Fifteen grams of agar have been used to the litre.

Krainsky's dextrose agar contains 10 g. dextrose, 0.5 g. NH_4Cl, with varying amounts of KNO_3, asparagin, or peptone to the litre (concentration of the agar not stated). In the present work 1.5 g. sodium asparaginate per litre has been used as the source of nitrogen; and 15 g. agar have been used per litre.

WAKSMAN AND CURTIS' WORK.

Waksman and Curtis, by the use of some of Krainsky's methods, have recently[21] added to the list of names by describing eighteen new species. Their descriptions are even more incomplete than Krainsky's. The broadest divisions recognized in their "key" are based upon liquefaction and pigment production in gelatin, both of which features vary too much with differences in the composition of the medium to be of value for classification. In this key, moreover, the finer divisions are based upon chromogenesis on some unnamed medium. This makes recognition of the species very difficult, because the slightest change in the composition of the medium often causes a great change in the appearance of the growth.

Probably the medium referred to in Waksman and Curtis' key is what they call "Czapek's agar," that being the medium used most extensively in their work. They do not give the formula of this medium nor any reference to an earlier publication of its formula. In a personal letter Waksman has given its formula, and it proves to be a modification of the saccharine nutrient solution described by Czapek.[22] They have added 0.2 per cent KNO_3 to this solution as recommended by Dox[23]; and have further modified it by substituting dibasic for monobasic potassium phosphate, which of course alters the reaction of the medium. To this solution they have added 1.5 per ct. agar.[24] Plainly, with all these modifications, it is incorrect to call it Czapek's agar. A solution of the same composition is referred to by Thom[25] as "Dox's solution." As Dox also used agar media which contained this nutrient solution, the medium used by Waksman and Curtis will be referred to here as Dox's agar.

This is the only agar medium which Waksman and Curtis definitely mention in their publication. They do not refer to the use

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[21] Waksman, S. A., and Curtis, R. E. The Actinomycetes of the soil. *Soil Science*, 1:99–134. 1916.

[22] Czapek, F. Untersuchungen über die Stickstoffgewinnung und Eiweissbildung der Pflanzen. *Beitr. Chem. Phys. u. Path.* 1:538–560. 1902. See page 542.

[23] Dox, A. W. The intracellular enzymes of Penicillium and Aspergillus. U. S. Dept. Agr., Bureau An. Indus., Bul. 120. 1910. See page 37.

[24] The formula as given in Waksman's letter is: 0.5 g. $MgSO_4$, 1 g. K_2HPO_4, 0.5 g. KCl, 0.01 g. $FeSO_4$, 2 g. $NaNO_3$, 30 g. sucrose, 15 g. agar, 1000 c. c. water.

[25] Thom, C. Cultural studies of species of Penicillium. U. S. Dept. Agr., Bur. An. Ind., Bul. 118. 1910.

Krainsky's media. This raises the question whether they were justified in establishing new species without having any direct means of comparing their cultures with Krainsky's. The difference between the growth of Actinomycetes on their medium and on Krainsky's is well illustrated in Table I (p. 169), in which the chromogenesis of six different cultures is given on Dox's agar and on Krainsky's malate agar. Four of these six cultures produced but scanty growth on Dox's agar, while growing vigorously on Krainsky's; and in several cases (see especially culture No. 3) the difference in appearance was so great that identification would plainly be impossible unless the culture studied were grown on the same medium as that which was used by the author of the species to be identified. Nevertheless Waksman and Curtis have stated that some of their cultures are identical with certain of Krainsky's species, presumably basing their judgment upon the fact that the appearance of the growth obtained upon Dox's agar is similar to that described by Krainsky as occurring on his own entirely different media. Plainly they are not justified in doing this, or in establishing new species without having studied the growth of their cultures upon Krainsky's media.

CLASSIFICATION.

METHODS USED IN THE PRESENT WORK.

Morphological criteria.— In the present work, the greatest stress has been laid upon cultural characteristics. There is reason to hope, however, that in a final classification the broadest divisions may be based upon differences in morphology. Neither Krainsky, nor Waksman and Curtis laid much stress on morphology in making their primary divisions. Krainsky divided the organisms into two divisions; — the macro-group which produces large colonies and has oval or spherical conidia, and the micro-group which produces small colonies and has spherical conidia only. Waksman and Curtis, probably correctly, disregard size of colonies. They base their primary divisions on liquefaction and pigment production in gelatin; and their secondary divisions on the production of spirals in the aerial mycelium. The former of these two characteristics, as already mentioned, is probably inconstant; but the second characteristic deserves further investigation. The shape of the conidia and the production of spirals in the aerial mycelium are both features that may serve as a basis of broad divisions. Before they can be used for this purpose, however, a thoro study of their constancy must be made. This point is being investigated at present, and it is hoped to use morphological features as a basis of primary divisions when the complete classification of the group is published. At present cultural characteristics alone have been investigated from this point of view; so in this preliminary classification most stress is laid on them.

Growth on glycerin media.— The greatest aid yet found in classifying these organisms has been the use of glycerin in synthetic media. The use of glycerin agar for stimulating their growth is not a new idea. It was used in the earliest studies on Actinomycetes; but apparently the early investigators added glycerin to the ordinary complex organic bacteriological media and so failed to obtain its full value in the separation of species. Krainsky, who seems to have been the first to use media of simple chemical composition for the purpose of classifying the Actinomycetes, did not use glycerin.

The mere addition of 1 per ct. of glycerin to Krainsky's calcium malate medium has been found to increase its value greatly as a medium for the differentiation of species. The number of different types of chromogenesis produced upon it is marvelous. In the three hundred cultures studied the following different colors of aerial mycelium have been observed: pink, orange, buff, gray-white, steel-gray, dark gray, green and blue. The following colors have been observed in the mass of growth itself: dark red, light red to pink, orange, deep brown, light brown, buff, brown and green mottled, gray to cream-color, yellow, yellow-green, dark green, blue, and black. The following pigments have been found diffused thru the medium: red, orange, brown (various shades), gray, yellow, green, and blue. As a different color is often found in each of these three places, the number of possible variations is very large. Fortunately the pigment production by any one strain is fairly constant. A large number of cultures have been reinoculated repeatedly and, altho there is some variation, the chromogenesis is sufficiently constant to serve to identify many of the types beyond question. The types which produce definite chromogenesis on malate-glycerin agar are regarded as fairly distinct; and probably only those types that do not produce definite chromogenesis upon this medium require further subdivision.

A substitute for malate-glycerin agar has been found which promises to give fully as good results. Malic acid is an expensive compound, and under present commercial conditions is almost impossible to obtain in this country. Citric acid is more easily obtained and is similar to malic acid in composition. An experiment therefore was performed to see if it could replace malic acid. Calcium citrate, however, is less soluble than calcium malate, so it could not be used. Instead, citric acid was used, and before adding the agar, was neutralized with the proper amount of sodium hydroxide. In order to compare this citrate-glycerin medium with the malate-glycerin medium, 79 cultures, of which 50 or 60 were of distinctly different types, were inoculated into both media. Forty-five, or a little over half, of the cultures grew alike on both media. Two of the other thirty-four cultures, altho different on the two media, were equally characteristic on both; 17 of them were more characteristic on the citrate agar, and 15 of them more characteristic on the malate agar.

From this it was concluded that sodium citrate was as valuable in the differentiation of species as calcium malate.

One other glycerin medium giving good results is the asparaginate-gylcerin agar referred to in an earlier paper of this series.[26] When soil is plated in this medium, more *Actinomyces* colonies develop than do on any of the other media yet investigated upon which the Actinomycetes produce characteristic growth. Its chief value, therefore, is for the isolation of *Actinomyces* cultures from soil, rather than for their characterization after isolation. There is often no similarity between the appearance of a certain culture growing on this medium and its appearance upon either of the two glycerin media just mentioned.

Growth on other media.— For the purpose of classification, the medium which has proved the most valuable of all investigated except the citrate-glycerin and malate-glycerin media is Krainsky's dextrose agar.[27] It has proved possible in several cases to distinguish two types from each other by means of their chromogenesis on this agar altho the growth of both is practically alike on malate-glycerin agar. The chief weakness of this medium is that it does not allow the cultures to produce vigorous growth; and as a result the characteristic features of an organism may not show up on it unless the culture used is in especially vigorous condition. It will probably prove possible in the future to modify the medium so as to obviate this disadvantage.

Dox's agar (see p. 164) has also been used in the present work. Like all other media of definite chemical composition, it has its advantages. Some types of Actinomycetes develop characteristics upon it that do not show on other media; but in general it does not compare with the glycerin media. Nearly all the cultures investigated grow upon it very poorly, even more poorly than upon Krainsky's dextrose agar. Apparently it is not so well suited to the growth of Actinomycetes as to that of higher fungi. As Waksman and Curtis' species, however, are characterized largely by means of their growth upon this medium, it must be used by anyone trying to identify their species.

Further aid in classification has been obtained by means of the test for nitrate reduction. The ordinary nitrate broth is useless for this purpose, because Actinomycetes grow poorly in it, if at all. Instead an ammonium nitrate medium [28] of Krainsky's was used. Tests for nitrite have been made after a few days' incubation at

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[26] Tech. Bul. 57, p. 25. Its formula is: distilled water 1000 c. c., agar 12 g., sodium asparaginate 1 g., dextrose 1 g., glycerin 10 g., $NH_4H_2PO_4$ 1.5 g., $CaCl_2$ 0.1 g., $MgSO_4$ 0.2 g., KCl 0.1 g., $FeCl_3$ trace. Reaction adjusted by the addition of 8 c. c. normal NaOH.

[27] See footnote 20.

[28] This medium contains 10 g. dextrose, 0.5 g. K_2HPO_4, and 0.5 g. NH_4NO_3 to the litre.

25° C. In some cases the results have proved of value, altho in others they have given inconsistent results. Further investigation of this test is being made.

Weakness of present methods.— The results obtained by this investigation of methods have shown the difficulties to be encountered in establishing species at present. Nearly all species so far described have been characterized largely by means of their color reactions on solid media.

Table I shows how great the variations in color reactions may be with slight changes in the composition of the medium. The three malate-glycerin media mentioned in that table differ only in containing respectively, calcium malate, sodium malate, and sodium malate with calcium chloride. If these salts ionize in the media, the differences should be of very little consequence. Nevertheless, the table shows that some of the cultures produced different color reactions on these slightly different media. Perhaps the most striking case of variation is shown by Culture No. 3, which produced a purple color in the mass of growth with formula 2 and 3, but not with formula 1, and a blue color within the medium in the case of formula 3, but not in the case of formula 1 or formula 2. These differences are not due to chance variation, as all of the tests listed in the table have been made twice at different times, and some of them three or more times, always with constant color reactions. It seems quite probable that equal differences might be produced by variations in the mineral salts occurring as impurities in commercial agar. For this reason one must be very cautious in using color reactions to characterize species of Actinomyces, basing no species upon them until after a thoro study of their constancy.

In the course of the present work it has proved impossible to predict the growth of a certain *Actinomyces* culture upon a new medium from a knowledge of its growth upon other previously investigated media. As a result, every new medium that has been tested has served to break up still further the types already recognized by the appearance of their growth on other media. This process is likely to continue for some time yet, as new media are investigated. A complete characterization of any species of *Actinomyces*, if dependent largely upon cultural characteristics, should include a description of the growth on a great variety of synthetic media.

THE MOST ABUNDANT TYPES IN SOIL.

About three hundred cultures have been isolated from the different soils investigated, and have been studied by the methods just mentioned. They have been classified provisionally into about seventy types. Most of these types were found only two or three times in the course of the work; but three of them proved to be fairly common. Descriptions will be given here of only these three types.

Table I.— Variations on the Chromogensis of Actinomycetes Produced by Differences in the Composition of the Media.

Colors produced by six selected strains of *Actinomyces*.

		Dox's agar.	Krainsky's agar.	Malate-glycerin-agars*		
				Formula No. 1.	Formula No. 2.	Formula No. 3.
Culture No. 1.	Mass of growth:	Colorless†	Colorless	Orange	Buff	Buff
	Aerial mycelium:	Buff	Pink (or buff) and white	Buff to orange	Gray-white	Buff to white
	Medium:	Brown, or colorless	Colorless	Orange-brown	Brownish	Yellow
Culture No. 2.	Mass of growth:	Buff	Buff	Gray to brown	Dark brown	Dark brown
	Aerial mycelium:	White	Gray and white	Gray-white	Pink and white	Pink and white
	Medium:	Pinkish brown	Uncolored	Faint brown	Deep red-brown	Deep red-brown
Culture No. 3.	Mass of growth:	Colorless†	Green to colorless	Dark green	Dark green to purple	Purple to blue
	Aerial mycelium:	......‡	Gray to white	Blue to gray	......‡	Blue and gray-white§
	Medium:	Uncolored	Green, or colorless	Dark gray-brown	Brownish	Brown and blue
Culture No. 4.	Mass of growth:	Colorless†	Buff to colorless	Buff to gray	Green	Green to colorless
	Aerial mycelium:	White§	White	Gray-white	Gray and white	White
	Medium:	Uncolored	Uncolored	Yellowish	Yellow-brown	Yellowish
Culture No. 5.	Mass of growth:	Gray	Buff	Pinkish brown	Gray to colorless	Buff to pink
	Aerial mycelium:	......‡	Gray-white	White	White	White
	Medium:	Uncolored	Uncolored	Pink	Light brown to colorless	Brownish
Culture No. 6.	Mass of growth:	Colorless†	Colorless	Colorless	Colorless	Colorless
	Aerial mycelium:	White	White	White	White and violet	White and violet
	Medium:	Uncolored	Uncolored	Uncolored	Brownish	Yellowish

* Of the malate-glycerin agars, formula No. 1 is the same as Krainsky's malate agar with the addition of 1 per ct. of glycerin. The only differences between formulae 1, 2, and 3 are as follows:
Formula No. 1 contains 5 g. calcium malate per litre;
Formula No. 2 contains 4 g. malic acid; 56 c.c. normal NaOH; 2 g. CaCl;
Formula No. 3 contains 4 g. malic acid; 56 c.c. normal NaOH; no calcium.

† Growth scanty.

‡ Aerial mycelium absent.

§ Aerial mycelium scanty.

The dark brown type. A. pheochromogenus, n. sp. This type is characterized by its thick, pure white aerial mycelium and by the production of a deep brown pigment which colors both the mass of growth and the medium. This brown pigment resembles that which characterized *A. chromogenus* Gasperini, but is produced in nearly all synthetic media as well as in gelatin and in agar containing peptone. The name *A. chromogenus*, might, therefore, be emended to fit the present species. It has seemed best, however, not to emend Gasperini's name, but to adopt the practice begun by Krainsky of placing a descriptive prefix before the adjective *chromogenus* in order to show that the species is included within the original description of *A. chromogenus*. The present species is not described by either Krainsky or Waksman and Curtis, altho a culture of it, unnamed, has been received from Waksman. About twenty cultures of it have been obtained in the course of the present work.

Morphology: Conidia are short rods with deeply stained granules, one at each pole.

Growth on gelatin: Liquefaction slow. Medium colored brown.

Growth on beef-extract-peptone agar. Mass gray to brown, aerial mycelium white to gray, but often absent, medium deep red-brown.

Growth on calcium malate agar: Without glycerin: mass buff to brown, aerial mycelium white, medium brownish. With glycerin: mass dark brown, vigorous, deeply rugose, aerial mycelium pure white, often rising 2 or 3 mm. above the mass of the growth, medium deep red-brown.

Growth on Dox's agar: Mass dark brown, aerial mycelium buff, medium deep brown.

Nitrate reduction in Krainsky's ammonium nitrate medium: doubtful.

The potato-scab type.— (Including *A. scabies* (Thaxter) and *A. diastatochromogenus* Krainsky.) This type does not have such definite cultural characteristics as *A. pheochromogenus*. Its growth on all media is gray to buff, with a dark gray aerial mycelium, (sometimes gray-white in less vigorous cultures). On synthetic media it rarely produces a pigment which diffuses thru the medium (or at the most the medium becomes faint yellow to brown); but on beef-extract-peptone agar and in gelatin the well-known brown pigment characteristic of "*A. chromogenus*" is produced. About 15 of the 300 cultures studied have these cultural characteristics. Of these 15, there are 8 that reduce nitrates in Krainsky's ammonium nitrate medium, 7 that do not. Among the 8 that reduce nitrate are 3 that were isolated from scabby potatoes. The other cultures came from soil.

The cultures that reduce nitrates agree, so far as can be determined, with *A. diastatochromogenus* Krainsky. The three that were isolated from potato scab, however, are undoubtedly the causal organism of that disease; and as Thaxter named that organism *Oospora scabies*, they must be called *A. scabies* (Thaxter). The pathogenicity of the cultures obtained from soil has not yet been tested, so it is not known whether they all belong to the species *A. scabies.* If not, the non-pathogenic forms should be called *A. distatochromogenus* Krainsky. Their pathogenicity not having been tested, they are grouped together for the present with the unquestionably pathogenic forms under the heading " potato scab type."

Recently Lutman and Cunningham [29] suggested reviving the name *A. chromogenus* Gasperini for the potato scab organism. In this they were obviously incorrect. If *O. scabies* and *A. chromogenus* are synonymous, Gasperini's name is correct, of course, on grounds of priority; but there is so much doubt as to which of the many species producing brown pigment in gelatin was studied by Gasperini, that the specific name used by Thaxter is not invalidated by the earlier name used by Gasperini.

Morphology: Conidia are short rods with deeply stained granules, one at each pole.

Growth on gelatin: Liquefaction rapid. Medium colored brown.

Growth on beef-extract-peptone agar: Mass gray or colorless. Aerial mycelium gray-white, white, or absent. Medium browned (sometimes only faintly).

Growth on calcium malate agar: Mass gray to buff to yellow or colorless. Aerial mycelium dark gray. If no glycerin is used, medium is uncolored; with glycerin, it is a faint brown or yellow. Growth more vigorous if medium contains glycerin.

Growth on Dox's agar: Mass buff or cream-color. Aerial mycelium gray to brown, or absent. Medium faint brown.

Nitrate reduction in Krainsky's ammonium nitrate medium: The typical cultures of *A. scabies* reduce nitrate vigorously. Seven of the soil cultures studied do not reduce nitrate, a fact which suggests that two distinct species may be included in this type.

The pink-spored type.— About 30 of the 300 cultures were found to belong to this type. It is characterized by the production of a gray-pink aerial mycelium on malate and citrate agars with and without glycerin. It does not agree with any of the species described by Krainsky or by Waksman and Curtis. There is considerable doubt as to whether it is a distinct species, so for the present it is not given a specific name.

Morphology: It is uncertain whether its conidia are round or rod-shaped. In the same preparation round coccus-like bodies are observed together with rods. The rods show deeply stained round bodies at the poles, which look like small cocci if separated. When coccus-like bodies are observed they are always in pairs. It is possible that the conidia are round, and are often bound together in pairs; or equally possible that the conidia are rod-shaped with deeply stained polar granules which look like separate conidia when the faintly stained substance between them is not visible.

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[29] Lutman, B. F., and Cunningham, G. C. Potato Scab. Vt. Agr. Exp. Sta., Bul. 184. 1914.

Growth on gelatin: Liquefaction fairly rapid. Medium colored brown.

Growth on beef-extract-peptone agar: Mass gray, aerial mycelium white to light gray, or absent, medium browned.

Growth on calcium malate agar: Colors about the same with and without glycerin; but growth is much more vigorous with glycerin. Mass cream-colored to uncolored, aerial mycelium gray-pink to brownish gray, medium uncolored if no glycerin is used, but browned if the medium contains glycerin.

Growth on Dox's agar: Almost nothing visible but aerial mycelium. Mass colorless, if present. Aerial mycelium salmon-pink; medium uncolored.

Nitrate reduction in Krainsky's ammonium nitrate medium: Sometimes present; sometimes absent. Perhaps this type may be broken up on the basis of nitrate reduction; but it has not been shown yet whether the test gives consistent results.

THE NUMEROUS LESS COMMON TYPES.

Besides the three fairly common types, numerous other types have been found in the soils studied, of each of which only a few cultures occur in the collection at hand. At present they are distinguished from each other by chromogenesis and form of growth on beef-extract-peptone agar, malate-glycerin agar, asparaginate-glycerin agar and Krainsky's dextrose agar. Some of them are more clearly marked types than the three mentioned on the preceding pages; but as they are less common in soil, no attempt will be made to describe them until the later more complete publication on the subject. There are twenty of these types of which only one culture each has been obtained in the course of the work. Of the others, seldom more than five or six cultures each have been obtained. The largest number of cultures of any one of these types in the whole collection of 300 cultures is eleven, and this particular type is distinguished by such indefinite characteristics that it may easily be broken up on further study.

RECOGNITION OF COLONIES ON PLATES.

Of the three most common soil types, only *A. pheochromogenus* can be recognized from its colonies on plates. If soil is plated on asparaginate-glycerin agar, this type can always be recognized, if present, by its deep brown colonies. The two other common types produce non-characteristic colonies on this medium, altho there are a number of the less common types that can be recognized by their colonies. It is important that some medium be devised upon which the common soil types can all be recognized, in order that plates made from soil may give an idea of the relative number of the different types present. Such a medium ought also to give a high total count. Unfortunately no such medium has yet been found.

Agar will undoubtedly prove a more satisfactory medium for this purpose than gelatin, as it is possible to have more control over its composition. Gelatin, like other complex nitrogenous material, tends to obscure the color reactions that are most characteristic of the various types. The one advantage of gelatin, so far as concerns the study of the Actinomyces flora of soil, is the ease with which colonies of Actinomycetes on it can be distinguished from those of the true bacteria. For this reason it has been used in the present work whenever it has been desired to obtain a comparison between the numbers of Actinomycetes and those of the true bacteria in soil.

DISTRIBUTION.

Occurrence in nature generally.— It is possible that Actinomycetes are as widely distributed in nature as true bacteria. As pathogens they seem to be less abundant; but saprophytic forms have been reported as occurring in many places under many different conditions, especially in air and soil. Their occurrence in many places may be from chance contaminations due to the fact that their conidia are very light and easily wind-borne; but soil seems to be their natural habitat. To anyone familiar with these organisms, the very odor of soil is proof enough of their abundance in it, as is the odor of some musty cellars proof that they live there.

The most striking fact in regard to their distribution is the great diversity of species. This fact has not been realized in the past, largely because they were studied upon media on which their characteristic features did not appear. Even Krainsky, with the use of special media, described only eighteen types. Neither the findings of Krainsky nor of any other investigator suggested the great diversity of species that has been observed in the course of the present work. At least sixty, and perhaps seventy-five, distinct types have been found in a collection of only about three hundred cultures. These cultures were obtained by the direct plating technic without preliminary cultivation or incubation at special temperatures. If a special search had been made for as many species as possible, there is no doubt but that the number of distinct types might have been doubled or even trebled. It is not impossible that the Actinomycetes are as rich in species and as diverse in function as the lower bacteria.

Occurrence in soil.— This group is one of the most abundant in soil. Its great abundance was discussed in a recent publication,[30] in which it was also mentioned that more *Actinomyces* colonies developed on plates from sod soil than on plates from cultivated soil. They were found to comprise about 20 per ct. of the total flora in cultivated soil and about 37.5 per ct. in sod soil.

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[30] Conn, H. J. A possible function of Actinomycetes in soil. N. Y. Agr. Exp. Sta., Tech. Bul. 52. 1916. *Jour. Bact.*, 1:197–207. 1916.

The difference between sod and cultivated soil in Actinomyces-content is usually indicated by the odor of the soil. The difference in odor is often so strong that even an inexperienced person can pick out the sod soil from a pair of unlabeled samples provided he is first allowed to smell of some *Actinomyces* culture with characteristic odor and is asked to select the soil which smells like the culture. An experienced investigator can make a fairly accurate guess as to the Actinomyces-content of a soil by means of its odor alone.

It was further shown in Technical Bulletin No. 52 that the numbers of *Actinomyces* colonies developing from soil tended to become larger, the longer grass had been growing upon the soil. This fact shows that the Actinomycetes may fluctuate in number in soil and indicates that they are active there. In this respect they are to be compared with the non-spore-forming rather than with the spore-forming bacteria. In interpreting plate counts of Actinomycetes, however, it must always be remembered that each colony probably arises from a conidium or from a chain of conidia, and therefore an increased plate count may mean merely an increased spore-production and not an actual increase in vegetative forms.

SIGNIFICANCE OF THE ACTINOMYCETES IN SOIL.

The common occurrence of Actinomycetes in soil has led to many speculations as to their significance. Ammonification, nitrate-reduction, and cellulose-decomposition have at one time or another been definitely stated to be the function of these organisms in soil. Generally, however, such conclusions have been drawn from observations in pure culture, which do not necessarily show what the activities may be under soil conditions. It is very likely that certain types of Actinomycetes may carry on the activities assigned to them; but only a comparatively few different varieties have been studied, and observations as to these few cannot logically be applied to the very diverse Actinomyces flora of soil.

The statement has sometimes been made that Actinomycetes have to do with the decomposition of manure. In this respect, the results of the experiment reported in Technical Bulletin No. 59 (p. 15) are rather surprising, for in that case the numbers of Actinomycetes developing on the plates remained almost constant after the addition of manure. Altho that experiment by no means disproves their activity in manure-decomposition, it emphasizes the fact that more work is needed before their part in such activities can be definitely determined.

It has also been noticed in the past that Actinomycetes are sometimes found in the roots of plants.[31] This, together with the fact that the cause of potato scab is an Actinomyces, suggested the idea mentioned in the writer's recent publication [32] that the reason for the larger numbers of Actinomycetes in sod soil is because they are concerned in the decomposition of grass roots. An experiment has recently been carried on to obtain further information on this subject. Some soil which had been fallow for four years and in which the Actinomyces-content was so low that only a little over 10 per ct. of the colonies on the plates belonged to this group, was brought into the laboratory and divided into two portions. One portion was mixed with some grass roots that had been obtained from sod by washing away the soil around them; the other portion was aerated to the same extent but otherwise untreated. Each portion was then divided into halves and each half put in a pot. These four pots were kept at room temperature, with water added at intervals so as to keep the moisture content at about 10 to 20 per ct. For convenience in sampling, the four pots were arranged in two sets, each set having one pot with and one pot without grass-roots. Set No. 1 was sampled more often than set No. 2; but the results from each set may be assumed to apply to the other also, because whenever both sets were sampled on the same day, similar results were obtained from both.

Gelatin plates were made from these pots at intervals and the results of the platings are shown in Table II. The figures in this table are in all cases the averages of at least three plate-counts. Two dilutions were always used and three or four plates poured of each dilution. Whenever the figures obtained by means of one dilution checked with those of the other, the individual plate counts were all averaged together; when they did not check, the average was obtained from the plates of that dilution giving between 50 and 100 colonies per plate (or nearest to these figures in case neither dilution gave plate counts within these limits). The left-hand half of this table gives the number of *Actinomyces* colonies per gram, with and without grass-roots, the right-hand half gives the total number of colonies on the plates. The arrangement of the left half of the table is the same as the arrangement of the left half of Table II in Technical Bulletin No. 52 (which compares the number of Actinomycetes in neighboring sod and cultivated spots); but the right half of the table is arranged differently. The right half of the table in Technical Bulletin No. 52 gave the percentage of Actinomycetes in the total flora; but in the case of the present pot experiment, percentage figures have little meaning because of the great increase in the total flora of the pot with grass-roots added (as shown in column 7). They are therefore omitted from the table.

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[31] See footnote 15.

[32] See footnote 30.

TABLE II.— ACTINOMYCETES IN DUNKIRK SILTY CLAY LOAM, WITH and WITHOUT ADDITION OF GRASS-ROOTS.

Figures based on number of colonies developing on gelatin plates.

TIME SINCE ADDITION OF GRASS-ROOTS	Set of pots	ACTINOMYCES COLONIES PER GRAM				TOTAL COLONIES PER GRAM	
		Grass-roots added.	Un-treated.	Differ-ence.	Ratio.	Grass-roots added	Un-treated.
FIRST ADDITION							
1 day..........	No. 1	3,000,000	2,200,000	800,000	....	38,000,000	28,000,000
6 days..........	No. 1	*1,700,000	?2,100,000	?	....	30,000,000	22,000,000
14 days..........	No. 1	6,000,000	3,500,000	2,500,000	1.7:1	80,000,000	30,000,000
23 days..........	No. 1	4,000,000	2,500,000	1,500,000	1.6:1	35,000,000	20,000,000
3 mo., 7 days....	No. 1	6,000,000	1,700,000	4,300,000	3.5:1	43,000,000	13,500,000
3 mo., 20 days....	No. 1	6,000,000	3,200,000	2,800,000	1.9:1	31,000,000	32,500,000
3 mo., 20 days....	No. 2	6,000,000	3,000,000	3,000,000	2 :1	29,000,000	23,000,000
4 mo., 7 days....	No. 1	5,300,000	2,000,000	3,300,000	2.6:1	33,000,000	19,000,000
4 mo., 15 days....	No. 1	6,300,000	3,500,000	2,800,000	1.8:1	23,500,000	24,000,000
4 mo., 15 days....	No. 2	4,500,000	3,100,000	1,400,000	1.5:1	22,000,000	18,000,000
7 mo., 15 days....	No. 1	6,200,000	2,900,000	3,300,000	2.1:1	30,000,000	16,000,000
7 mo., 15 days....	No. 2	8,000,000	4,000,000	4,000,000	2 :1	32,500,000	28,000,000
10 months.......	No. 1	5,000,000	2,500,000	2,500,000	2 :1	14,000,000	8,000,000
10 mo., 7 days....	No. 2	4,300,000	2,700,000	1,600,000	1.6:1	20,000,000	9,500,000
Average†........	......	5,500,000	2,900,000	2,750,000	2.03:1	....	....
SECOND ADDITION‡							
4 days..........	No. 2	*2,300,000	?1,800,000	500,000	*1.3:1?	75,000,000	7,000,000
23 days..........	No. 2	12,500,000	2,000,000	10,500,000	6.3:1	130,000,000	10,000,000
3 mo., 20 days....	No. 2	7,500,000	2,500,000	5,000,000	3 :1	37,000,000	11,500,000
5 months........	No. 2	10,000,000	1,800,000	8,200,000	5.5:1	65,000,000	14,500,000
6 months........	No. 2	7,000,000	1,600,000	5,400,000	4.5:1	46,000,000	15,000,000
Average†........	......	9,200,000	2,000,000	7,300,000	4.8:1	....	....

* These counts are inexact because on this date the plates made from the pot with grass-roots liquefied too rapidly to permit a satisfactory count.

† The figures obtained during the first week after the addition of grass-roots are omitted from this average.

‡ The second lot of grass-roots was added to the previously treated pot of set No. 2 ten and half months after adding the first lot.

As shown in this table, the Actinomyces-content of the untreated pots remained almost constant thruout the experiment, averaging 2,900,000 colonies per gram during the first ten months. The Actinomyces-content of the pot with grass-roots, however, increased to 6,000,000 in two weeks and remained at about that height during the ten months (average 5,500,000 per gram). The average difference in Actinomyces-content between the two soils after the first two weeks was 2,750,000. The average ratio of the Actinomyces-content of the soil with grass-roots to that of the untreated soil was 2.03:1. Similarly, in Technical Bulletin No. 52 (Table II), in 34 comparisons of sod and cultivated spots of various soil types, the average ratio is 2.15:1. This similarity suggests that dead grass-roots mixed with soil cause the same stimulation to the Actinomycetes as brought about by grass growing on the soil.

It must be noticed, however, that the Actinomycetes are not the only organisms stimulated by the presence of the grass-roots. Fourteen days after the addition of the roots the total count was 80,000,000, while in the check pot it was only 30,000,000. Nearly all of this increase (altho this fact is not brought out by the data given in Table II) was due to an increase in the number of non-spore-forming bacteria in the soil with grass-roots, the number of spore-formers remaining practically constant thruout the experiment. The number of non-spore-forming bacteria remained higher in the soil with grass roots than in the untreated soil until about four and a half months after the treatment. This indicates that non-spore-formers, as well as Actinomycetes, take part in the decomposition of grass-roots, a fact still further brought out by the data in lower section of the table.

In the lower section of the table are given some further figures obtained by plating the soil in the pots of set No. 2 in the above experiment. Ten and a half months after beginning the experiment, one of these two pots containing grass-roots was further treated by adding a second, and larger, lot of grass-roots. On the same date the soil in the other pot of this set was also aerated and then returned to the pot without further treatment so as to serve as a check. It was found that the aeration alone did not affect the Actinomyces-content or the total count of the check pot; but that 23 days after treatment, the soil with grass-roots showed a count of 12,500,000 Actinomycetes per gram, six times that of the check pot. The total count had meanwhile increased to the unusually high figure of 130,000,000 per gram. As the spore-forming bacteria remained constant in numbers, it is evident that the high total count must have been due largely to a great increase in non-spore-forming bacteria. In other words, both Actinomycetes and non-spore-formers increased in number after the addition of grass-roots, and presumably both groups took part in their decomposition. Later the non-spore-forming bacteria decreased somewhat altho by no means to their original numbers, while the Actinomycetes remained fairly constant in number and about five times as abundant as in the check pot.

Two explanations of these results are possible. On the one hand it may be assumed that the non-spore-formers are more active in the early stages of the decomposition of grass-roots, the Actinomycetes in the later stages; or on the other hand the explanation may be that both groups multiply at first, and then later decrease in activity, but that no decrease in *Actinomyces* colonies is observed because of the comparative longevity of their conidia.

Some information on this point has been obtained from another experiment. Sod soil, showing a high plate count of Actinomycetes, was air-dried, and sifted thru a fine sieve so as to remove all but the smallest fragments of roots, then placed in a pot, brought up to its original moisture content and later watered at intervals. When it was last sampled, fourteen months after beginning the experiment, the Actinomycetes were still as high in numbers as in a check pot (from which the grass-roots had not been removed) and still comprised 40 per ct. of the total flora developing on the plates. This indicates that a soil may continue to show a high plate count of Actinomycetes for some time after the condition which stimulated their numbers has ceased to affect them. It suggests that their high numbers in sod soil may be due in part to the fact that their conidia are comparatively long-lived.

In brief, the experiments carried on to investigate the relation of Actinomycetes to grass-roots are inconclusive. They merely indicate that these organisms, like the non-spore-forming bacteria, take active part in some of the processes going on in normal soil, and are not inactive under such conditions, like the spore-forming bacteria.

GENERAL DISCUSSION OF THE WHOLE SERIES OF SOIL FLORA STUDIES.

The chief fact emphasized by these soil flora studies is the meagerness of present-day knowledge in regard to the micro-organisms of soil. The bacteria that have been shown to carry on nitrogen transformations and other activities known to occur in the soil and have therefore been given the most thoro study, do not seem to be especially numerous in soil, and it has never been shown that their numbers in soil increase while the activities ascribed to them are taking place. On the other hand, the bacteria that are numerous in soil and do multiply at times when great bacterial activity should be expected are organisms concerning which almost nothing is yet known.

One of the first steps necessary in order to put the knowledge of soil bacteria on a scientific basis is classification. It is difficult to make definite statements about soil bacteria without definitely described species. Statements frequently found in the literature in regard to the activities of certain soil micro-organisms are almost valueless to anyone except their author, because of the uncertainty as to the identity of the organisms studied. This confusion can be prevented in the future only by the development of a classification of the soil micro-organisms based upon the accumulation of thoro characterizations of the common species.

The species of soil bacteria that have been most thoroly characterized in the past are certain spore-forming bacteria. The spore-formers, however, are not so abundant or apparently so important as the other groups of organisms developing on the plates. Among these other groups — the non-spore-forming bacteria and the Actinomycetes — very few species have ever been thoroly characterized.

The difficulties to surmount in characterizing species among these organisms are great. Morphology has proved a great help in classifying the spore-formers; but morphological characters are of little help in the classification of the non-spore-formers, and they have not yet been successfully used in classifying the Actinomycetes. Physiological tests have been the basis of classification of certain kinds of bacteria (the *B. coli* group, for instance); but the ordinary physiological tests used to characterize bacterial species are not applicable to the majority of soil bacteria.

For this reason but few of the types described in this series of bulletins can be considered as species. Further classification must be undertaken, and the efforts of no one man will be enough to put the matter on a sound basis. It must be remembered, moreover, that systematic studies of soil bacteria are no more important than physiological studies. Neither is of much value without the other, and the results of one should be used to interpret the results of the other.

Physiological and systematic studies both depend upon cultural methods and upon the assumption that the organisms investigated are the active organisms in soil. There is need of some direct method of investigating the soil micro-organisms that can be used to check up the cultural methods. Within the last few months the writer has called attention to a method of using the microscope for the direct examination of bacteria in soil.[33] The weakness of this microscopic method lies in the fact that it is not possible to isolate the organisms thus revealed and there is no way of studying their activities. Nevertheless the method promises good results if used in conjunction with plate culture, and in continuing these flora investigations microscopic findings are to be compared with those obtained by cultural methods.

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[33] Conn, H. J. The direct microscopic examination of bacteria in soil. *Abstr. of Bact.,* 1: No. 1. 1917.

FOREWORD.

For many years the Official Dairy Instructors Association, which has recently become the American Dairy Science Association, has maintained a committee on dairy score card, which committee is responsible for the so-called " official " dairy score card. In 1912 this committee recognized the necessity for a different score card evaluating the quality of milk rather than the conditions under which milk was produced, and formed a subcommittee to study this problem. This subcommittee was later made an Association committee on milk quality. Extensive investigations have been conducted, particularly at the New York Agricultural Experiment Station and the Illinois Agricultural Experiment Station, at Urbana, in connection with this study, and the present publication is an analysis of the problem of milk quality as it appears to this committee after these years of study.

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WHAT IS MEANT BY "QUALITY" IN MILK.*

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INTRODUCTION.

Milk is often called good or bad, but it is difficult to define either quality. It needs but a slight study of the milk question to recognize that the goodness or badness of milk depends upon several factors. Because of the complexity of this situation there is much confusion in the public thought regarding quality in milk. As a result, milk is still commonly sold without the use of grades to designate quality, the State of New York being one of the few exceptions to this rule.

The present publication is a brief summary of previous considerations of the various sides of this question, a plea for a broader consideration of the problem of milk quality, and a suggestion regarding the line along which future progress in the improvement of city milk supplies will undoubtedly be made.

ELEMENTS OF QUALITY IN CITY MILK.

Many factors combine to determine the quality of milk. Each of the factors has been recognized as important at one time or another, but apparently thus far no one has succeeded in so fully analyzing the city milk situation as to formulate a complete expression for milk quality. The following summary[1] of the elements of quality in city milk under the headings of food value, healthfulness, cleanliness, and keeping quality is an attempt at such an analysis. The order of presentation of these elements is essentially that in which they have been previously brought to public attention.

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[1] This summary of elements of quality in city milk does not consider the occasional occurrence in milk of disagreeable substances, of which onions and gasoline are the most common examples, because the evident presence of such substances automatically excludes such milk from the city trade.

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FOOD VALUE.

While milk is sometimes used as a beverage, the fundamental reason for the existence of the present vast traffic in milk is the fact that milk is one of our most important foods. Not only does it offer energy in a readily available form, but the amount and variety of the compounds contained in milk make it a peculiarly valuable food for growing children. The present consumption of milk in this country is only about 0.6 pint per capita per day, altho from the standpoint of protein, which is especially needed by the growing child, or from the standpoint of total energy as utilized by the adult, much more food value is obtainable from milk for a given sum of money than can be purchased in any comparable food. The high food value of milk is shown by the following table recently prepared by the U. S. Department of Agriculture:

PROTEIN	ENERGY
1 quart of milk is equal to:	1 quart of milk is equal to:
7 ounces of sirloin steak	11 ounces of sirloin steak
6 ounces of round steak	12 ounces of round steak
4.3 eggs	$8\frac{1}{2}$ eggs
8.5 ounces of fowl	10.7 ounces of fowl

In 1856 the laws of Massachusetts[2] attempted to protect milk from adulteration and since that time federal, state, and municipal authorities have enacted laws establishing standards for butter fat and the other solids in milk. It was the original conception that milk is of essentially fixed composition and that the establishment of minimum standards would stop the watering and skimming of milk. The establishment of these legal standards undoubtedly has had a pronounced effect in limiting open and gross adulteration of milk, but the secondary and unexpected effects of such enactments have been such as to raise the question whether, taken as a whole, they have been beneficial to the quality of the milk supply.

While it is true that these legal standards set definite limits to the extent to which the food value of milk could be reduced without incurring the penalty of the law, at the same time they offered indirectly a stimulus for the reduction of such food value to a figure approximating these legal minimum standards.

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[2] Parker, H. N. City Milk Supply, p. 370. 1917.

The cost of producing milk at the farm is fairly proportional to the amount of food value in the milk. With the narrow margin of profit which exists in milk production, there has been a strong impelling force toward the production of milk with the smallest food value that the market would accept without reduction in price. When the law prohibited the reduction of food value by the direct addition of water, the same result was frequently accomplished by the selection of animals producing milk which approached or even fell below the legal minimum limits. It is a matter of common knowledge that the milk supplies of our larger cities have been falling in food value, and today much of the milk sold in such cities is almost exactly at the legal limit of fat and below the legal limit in solids not fat.

This reduction in food value is all the more striking in view of the marked preference which the consuming public has for milk of high food value. Many progressive milk dealers, recognizing this situation, have offered milk high in fat content at an advanced price with commercial success.

While there is no simple, and at the same time, entirely satisfactory method of expressing the food value of milk, it may be roughly measured in a variety of ways. The housewife customarily judges the food value of milk by noting the depth of the cream line in the milk bottle. The food values of the other constituents of normal milk do not vary in absolute proportion to the fat, and therefore the fat is not an entirely accurate measure of the food value of the milk; but, at the same time, the variations in total food value are so nearly proportional to the variations in the fat of the milk that the fat content of milk of cows may well be used as an index of the relative food value of various samples of milk. This index has the added convenience of being easily and accurately determined by means of the Babcock test.

HEALTHFULNESS.

It is not enough that a bottle of milk shall have abundant cream in order to be accurately characterized as good milk. If such milk should contain even a limited number of virulent typhoid-fever organisms, it would be rejected by anyone who was acquainted with this fact. While the milk business is conducted primarily because milk is a valuable food, the occasional appearance of an epidemic spread by the use of milk has made the public suspicious of the healthfulness of all milk. This public suspicion is a severe handicap to the milk business, and any procedure which will remove this suspicion and stimulate the increased consumption of milk will be of great economic benefit to the dairy industry as well as to the consumer.

While the possibility of milk functioning as a carrier of disease had been previously discussed, beginning about 1893[3] the use of the tuberculin test revealed a large amount of tuberculosis in dairy cattle and the public was impressed with the danger of spreading tuberculosis thru the milk.[4] Later investigations, particularly those made during the past fifteen years, have fully demonstrated the danger of tuberculosis being transmitted from cows to children thru the milk. Occasional epidemics[5] of septic sore throat and typhoid fever and less frequently epidemics of scarlet fever and diphtheria transmitted in the same way have given good grounds for suspicion regarding the healthfulness of the ordinary raw milk supply. The amount of danger from this source is commonly overestimated, but its existence, particularly in the case of children, is beyond question and should not be overlooked.

Health authorities, early recognizing tuberculosis of cattle as a public menace, attempted to stamp it out by the widespread application of the tuberculin test. The difficulties encountered in such an attempt made it evident that whatever may be the value of the tuberculin test as such, there is little prospect that the application of the test will become so widespread as to offer protection to the general milk supply.

It has also been recognized that tuberculosis is only one of a number of diseases which may be distributed thru the milk supply. Any plan which is to make milk a safe article of food must take account, not only of diseases which may be transmitted from the cow, but also of the more formidable list of diseases which may be transmitted by the milk from the people who handle it to those who consume it. The history of certified milk has made it evident that a careful medical supervision of both animals and men will reasonably protect the milk from danger of transmitting human diseases, but the expense of such supervision is large.

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[3] Pearson, L. A. Proceedings of the First International Veterinary Congress of America. Oct., 1913.

[4] Park, W. H., and Krumwiede, C. The Relative Importance of the Bovine and Human Types of Tubercle Bacilli in the Different Forms of Tuberculosis. Collected Studies from the Research Laboratories, Department of Health, New York City, **7:** 88-92. 1913.

[5] Trask, J. W. Milk as a Cause of Epidemics of Typhoid Fever, Scarlet Fever, and Diphtheria. Public Health and Marine Hospital Service of the United States, Hygienic Laboratory Bul. 56, pp. 23-149. 1909.

Kelley, Eugene R. The Quantitative Relationship of Milk-borne Infection in the Transmission of Human Communicable Diseases. *Jour. Am. Med. Assoc.* **67:** **1997-1999. 1916.**

Pasteurization of milk was early advocated as a means of safeguarding the consumer from the dangers, not only of tuberculosis, but of other transmissible diseases. As with the tuberculin test, so with pasteurization, many practical difficulties were encountered in applying the process to the milk supply.

The studies of Theobald Smith[6] and of Russell and Hastings[7] which pointed out the practicability of pasteurizing milk at 140° F. for thirty minutes, mark the real beginning of modern successful milk pasteurization. This pasteurization, which both gives the desired protection against disease germs and furnishes a product satisfactory to commercial milk requirements, was the beginning of a widespread general interest in the subject. This interest has grown to the point where the regulations of the largest cities and of some of the smaller cities make such pasteurization of the general milk supply compulsory. In some instances this movement toward pasteurization has even taken the form of state enactment.[8]

It is evident that if the milk supply is to be made so safe as to banish the suspicion of danger from disease germs, which is now a factor limiting the consumption of milk, the milk must either be produced under a careful medical supervision regarding the health of the cows and men or it must be properly pasteurized.

CLEANLINESS.

In order to conform to the general opinion of a good milk, it is not sufficient that a milk shall have high food value and shall be free from danger of disease. If at the bottom of the bottle of milk there is a distinct sediment, the purchasing public will uniformly reject the milk as being of poor quality. The public is justly desirous of having a clean food supply, and there is probably no food product regarding which it is more sensitive than milk. The extreme sensitiveness of the public in this matter is due in part to the fact that milk naturally lends itself to careful inspection. The white milk forms a natural background against which any foreign matter stands out with startling distinctness. As a result of these physical conditions the unaided eye is able to detect the presence of foreign matter in milk when it is present in such minute quantities as to practically defy detection by analytical methods. The sensitiveness of this inspection is shown by the fact that it is possible thus to find traces of foreign matter in practically any quart of milk which is critically examined, regardless of the care exercised in its production. In the certified milk from the cleanest dairies in the country which is annually brought together in competition at the National Dairy Show, such foreign matter is evident to the eye in over 80 per ct. of the bottles. On the other hand, the amount of this foreign matter is so slight in all certified milk, and in practically all commercial milk, as to be upon the very margin of detection by analytical methods.

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[6] Smith, Theobald. The Thermal Death-Point of Tubercle Bacilli in Milk and Some Other Fluids. *Jour. Exp. Med.* 4 : 217-233. 1899.

[7] Russell, H. L., and Hastings, E. G. Thermal Death Point of Tubercle Bacilli under Commercial Conditions. Wis. Agr. Exp. Sta. Ann. Rpt., 17, pp. 147-170. 1900.

[8] California St. Bd. of Health. Special Bul. 13. 1916. Oregon Dairy and Food Bul. 5, p. 2. 1917.

Taking advantage of the sensitiveness of the eye to differences in color, a method called the sediment test[9] has been devised for determining the cleanliness of milk. In applying this sediment test, measured quantities of milk are passed thru cotton and the dirt is observed as a residue upon the white cotton. This test has been quite widely applied in commercial work. While in rare cases the presence of considerable amounts of dirt has been demonstrated, in practically all instances the amount of dirt found in the milk has been slight. When attention has been directed to the presence of any considerable quantities of dirt, the conditions of milk production have been promptly modified so as to bring the milk to a uniformly high standard of cleanliness.

Milk as it is now generally produced and handled is one of our cleanest foods.

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[9] Weld, I. C. A plan for Improving the Quality of Milk and Cream Furnished to New Hampshire Creameries. N. H. Agr. Exp. Sta. Bul. 132. 1907.

KEEPING QUALITY.

In order that a milk shall be justly entitled to be called good milk, it is not sufficient that it be high in food value, that it be free from danger of carrying disease, and that it be clean, because if it is sour when delivered to the consumer or sours promptly thereafter, it is unsatisfactory. In the northern states, at least, the delivery of milk once a day is expected to supply the needs of the family for the succeeding twenty-four hours. Accordingly, good milk must remain sweet during that period, and preferably during a longer period, in order to justly entitle it to be called good.

It is possible to estimate the condition of the milk with regard to food value, healthfulness, and cleanliness, by comparatively simple methods. The situation with regard to keeping quality is more complex. Souring is induced by the growth in the milk of minute forms of plant life — bacteria. This plant life attacks the sugar of the milk, using it as a food, and producing acid as a by-product. When the accumulation of this acid amounts to approximately 0.3 per ct., the milk begins to taste sour; and when the accumulation of acid has reached approximately 0.7 per ct., the milk curdles. The problem of maintaining a satisfactory keeping quality is essentially a problem of restricting the development of germ life. It is possible to meet this problem either by preventing the entrance of germs, by destroying them after they enter, or by holding the milk under conditions which will prevent the activity of the germs after they enter.

While the problem of the keeping quality of milk can thus be stated in simple terms, the actual restriction of contamination and of development of germ life is a complex matter. There is still a lack of knowledge regarding the relative importance of the various avenues thru which bacteria gain access to the milk, and this results in a lack of knowledge regarding the most practicable means of preventing their entrance.

In attempting to control keeping quality, various cities have made regulations establishing a maximum number of germs permissible in their milk supplies.[10] These regulations did not attain the desired results, and in many cases the cities further stipulated various conditions which must accompany milk production. The establishment of bacterial standards placed upon the milk producer and the milk dealer the responsibility of translating these standards into terms of dairy processes, while the detailed recommendations formulated by the health authorities are an attempt on their part to make this translation. In practice both these attempts have failed to accomplish the desired end. As a measure of the keeping quality there are many advantages in a direct[11] determination of the germ life, but this is a technical process not readily available to the dairymen and accordingly has certain limitations.

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[10] Bacteriological Standards for Milk. *U. S. Public Health Reports* 29 : 1218-1221. 1914.

The true measure of the keeping quality of milk is the time which elapses before it actually sours. This is the measure employed by the consumer, but manifestly it cannot be applied in advance at any earlier stage in the commercial life of the milk. A modification of this is possible in that samples of the milk under consideration may be held at high temperatures and the interval before curdling noted. From a comparative study of the effect of a temperature on germ growth, it would then be possible to translate this interval into the time which would elapse before the original milk would sour at the lower temperature at which it would normally be held. This procedure involves some time and technical apparatus which is not often available.

The commercial milk men have long employed the acid test, as well as their trained sense of taste and smell, in estimating the probable keeping quality of milk as delivered at their plants. By these means they have been able to anticipate somewhat the time at which milk will be no longer acceptable to the whole-milk trade, but it is only as milk approaches this limit that its condition is determinable by these means.

During the past few years there have been suggested a number of technical milk tests more or less closely related to keeping quality, such as the reductase test, the Schardinger reaction, the alcohol test, the catalase test, and the hydrogen ion concentration. In general the availability of these tests seems limited because they are mainly useful only in the later stages of the commercial life of milk.

In view of this unsatisfactory condition of knowledge regarding the measurement and control of the keeping quality of milk, the

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[11] Breed, R. S., and Brew, J. D. Counting Bacteria by Means of the Microscope. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 49. 1916.

New York [12] and Illinois [13] Agricultural Experiment Stations have undertaken extended and detailed study of the various factors affecting the entrance and growth of germ life in milk.

COMPLEXITY OF THE PROBLEM.

The above outline of the various phases of milk quality brings out the fact that at various times the students of the milk question have been interested first in one, then in another element of milk quality, and that in connection with each such attempt they have succeeded in devising a more or less successful index of quality with regard to the particular point under observation.

This publication is designed to emphasize the fact that the quality of city milk is not a simple matter to be adequately expressed after a consideration of any one factor, but that it is a complex matter which can be expressed only after an adequate consideration and evaluation of each of these four essential factors; namely, food value, healthfulness, cleanliness, and keeping quality.

While the percentage of fat in milk is not a perfect measure of the food value, it is an easily determined index of food value. While medical supervision of the health of the cows and the men or proper pasteurization are not absolutely self-sufficient guarantees of the healthfulness of milk, they are the most practicable and easily applied indices of healthfulness. The sediment test, while open to some objections, is a simple and easily applied index of milk cleanliness. The problem of a satisfactory index for keeping quality is not so simply solved. Among the many available tests, that one must be selected which will best suit the purpose in hand.

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[12] Harding, H. A., Wilson, J. K., and Smith, G. A. Milking Machines: Effect of Method of Handling on the Germ Content of Milk. N. Y. (Geneva) Agr. Exp. Sta. Bul. 317. 1909.

Harding, H. A., Wilson, J. K., and Smith, G. A. The Modern Milk Pail. N. Y. (Geneva) Agr. Exp. Sta. Bul. 326. 1910.

Harding, H. A., Ruehle, G. L., Wilson, J. K., and Smith, G. A. The Effect of Certain Dairy Operations upon the Germ Content of Milk. N. Y. (Geneva) Agr. Exp. Sta. Bul. 365, pp. 198-233. 1913.

Harding, H. A., and Wilson, J. K. A Study of the Udder Flora of Cows. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 27. 1913.

Ruehle, G. L. A., and Kulp, W. L. Germ Content of Stable Air and Its Effect upon the Germ Content of Milk. N. Y. (Geneva) Agr. Exp. Sta. Bul. 409, pp. 418-471. 1915.

[13] Prucha, M. J., and Weeter, H. M. Germ Content of Milk: I. As Influenced by Factors at the Barn. Ill. Agr. Exp. Sta. Bul. 199. 1917.

Much of the confusion in the public mind regarding milk quality has been due to a failure to discriminate properly between germ content and healthfulness, on the one hand, and germ content and cleanliness, on the other.

The introduction of the public to the subject of germ life came thru the attention which was early given to germs as producers of diseases such as tuberculosis[14] and typhoid fever. To the public, bacteria and disease became practically synonymous words. Later the attention of the public was directed to germ life in milk[15] at about the same time that its attention was directed to the possibility of germs of tuberculosis[16] being present in milk. Therefore, it is not at all strange that in public thought germ life and unhealthfulness of milk should have seemed identical.

Early in the present century Metchnikoff[17] and other writers began to lay stress upon the health-giving qualities connected with certain germs in milk, as those of the Bulgaricus group. More recently extensive commercial use has been made, not only of cultured milks of various kinds, but also of vast quantities of buttermilk containing the ordinary sour-milk organisms with or without the addition of cultures of the Bulgaricus forms. There is a continued satisfactory use of these sour-milk drinks which contain many millions or billions of bacteria per cubic centimeter, not only of these special organisms with foreign names, but also of the organisms present in our sour milk of commerce. These experiences are gradually bringing home to the public an appreciation of the fact that there is very little connection between the amount of germ life in milk and the healthfulness of milk.

The confusion in the public thought between the presence of germ life and cleanliness arises from the fact that it was originally believed that the seeding of milk with bacteria came about primarily as a result of a large quantity of bacteria being carried into the milk upon various forms of foreign matter, such as dirt and dust. Each particle of dust in the barn air was looked upon as an omnibus overloaded with attached germ life.

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[14] Koch, R. Die Aetiologie der Tuberkulose. *Mitt. aus dem Kaiserl. Gesundheitsamte* 2 : 1-88. 1884.

[15] Sedgwick and Bacthelder. A Bacteriological Examination of the Boston Milk Supply. *Boston Med. and Surg. Jour.* 12 : 25–. 1892.

[16] Russell, H. L. The Infectiousness of Milk from Tuberculous Cows. Wis. Agr. Exp. Sta. Ann. Rpt. 11, pp. 196-200. 1895.

[17] Metchnikoff, E. The Prolongation of Life. 1908.

More recent studies have shown that dust particles, instead of being loaded in the manner described, are actually in all but less than one case out of a hundred entirely free from any living organisms.[18] While it is true that small numbers of germs are carried into the milk upon dirt, the amount of dirt actually finding its way into the milk is so small in proportion to the mass of the milk that the germ life added in this way is relatively insignificant. The confusion regarding bacteria in milk is being cleared up by studies which show that the real source of contamination of milk is either an unusual population of bacteria in the udder, or far more frequently, the presence of a surprisingly large amount of germ life upon the utensils in which the milk is handled. So persistent is this idea of the constant association of germ life and dirt that the natural inference would be that utensils carrying large numbers of germs were dirty. This inference is not in accord with the carefully observed facts, since germ life is present in vast numbers upon dairy utensils which have been rendered clean in the ordinary sense of the word, but which have not been so handled as to obliterate germ life.

Later studies of germ life in the udder [19] have made it plain that germ life is constantly present in all samples of normal milk from the time it is secreted by the glands of the udder to the time it is utilized by the consumer.

Too frequently the public thinks of milk merely as a fluid containing butter fat, while it should of course recognize the fact that milk also normally contains about 5 per ct. of milk sugar, as well as varying amounts of nitrogenous substances which become most prominent in such things as cottage cheese. Until a few years ago, few people appreciated that in the process of milk secretion, worn-out gland cells and blood corpuscles are thrown off into the milk and form a part of normal milk, since they are uniformly and regularly present in considerable numbers in all milks.[20] The recentness of our appreciation of the normal presence of these cells in milk is shown by the fact that up to a few years ago in certain cities there existed regulations forbidding the presence of what are now known to be fairly normal quantities of these cells in milk.

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[18] Compare, for example, the number of dust particles per cubic foot of air as reported on page 61 of Final Report of the Committee on Standard Methods for the Examination of Air (*Am. Jour. Pub. Health* 7 : 54-72, 1917), where the number of dust particles per cubic foot of the air of New York City streets is given as between 400,000 and 1,000,000, as determined by the filtration method, with the number of bacteria per cubic foot of air as reported by Winslow, C. E. A., and Browne, W. W. (The Microbic Content of Indoor and Outdoor Air. *Monthly Weather Review* 42 : 452-453, 1914). The average numbers of bacteria which the latter authors report do not exceed 113 per cubic foot for air from the open country, from city streets, from offices, from factories, and from schools.

[19] Ward, A. R. The Invasion of the Udder by Bacteria. N. Y. (Cornell) Agr. Exp. Sta. Bul. 178. 1900.

Harding, H. A., and Wilson, J. K. A Study of the Udder Flora of Cows. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 27. 1913.

Evans, Alice C. The Bacteria of Milk Freshly Drawn from Normal Udders. *Jour. Infect. Dis.* 18 : 437-476. 1916.

Sherman, J. M. Studies on the Production of Sanitary Milk. Ann. Rpt. Penn. State College for 1914-15.

While the public is generally aware of the fact that milk always contains considerable quantities of germ life, it has probably not yet come to appreciate the fact that germs in milk are just as constant, and therefore just as normally a part of milk, as are milk sugar, fat globules, and body cells. The consumer has little interest in the germ content of milk except for a limited number of disease-producing forms against which he has a right to insist upon adequate protection, and except in so far as the germ life produces objectionable changes such as souring or bad flavors in the milk itself.

COMPOSITE EXPRESSIONS OF QUALITY.

As has already been stated, the students of milk have rcognized more or less distinctly the various elements of milk quality. However, the public mind insists upon a simple, direct statement of quality regardless of the complex relationship involved. It insists that a milk must be *good*, *medium*, or *bad*. Various plans have been devised for meeting this demand and furnishing a composite expression for milk quality.

CERTIFIED MILK.

Certified, as applied to milk, signifies that it has the food value of normal 4-per ct. milk, the healthfulness resulting from a careful medical supervision of all animals and men connected with the production and handling of the milk, the cleanliness following careful attention to the cleanliness of the animals and the utensils, and the keeping quality to be expected of fresh milk with a low germ content, kept at a very low temperature.

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[20] Breed, R. S. Cells in Milk Derived from the Udder. N. Y. (Geneva) Agr. Exp. Sta. Bul. 380. 1914.

SCORE CARD.

As a measure of the desirability of the ordinary milk supply, various dairy score cards have been suggested. These score cards are an attempt to express on a percentage basis the protection given milk on the farm from the danger of contamination with disease-producing germs both from animals and from men (healthfulness); the protection given milk from dirt (cleanliness); and the protection given milk by care of utensils, by cooling, and by prompt delivery (keeping quality). These score cards have uniformly failed to take account of the food value of the milk. Since these score cards are arranged on the basis of the agricultural methods and equipment rather than on the basis of the milk, it is but natural that in the cards themselves there should be much confusion regarding the items which apply respectively to healthfulness, cleanliness, and keeping quality. Some have expected to find correlation between germ content and the dairy score; others feel that there should be a correlation between cleanliness of the milk from dairies and the dairy score; while others expect a correlation between the score of the dairy and the presence or absence of disease germs. Such comparisons on the basis of a single element of equality are necessarily unfair to the score card unless it is recognized that the dairy score combines factors connected with all three elements of quality.

The essential difficulties of present score cards arise from the fact that they are an attempt to evaluate the influence of dairy environment and processes upon the milk, when the relative importance of such factors has not yet been sufficiently determined.

Grade.— This presentation would be very incomplete if it did not include a suggestion as to the manner in which the four elements of milk quality herein discussed might be combined so as to form a basis for defining grades of milk. The following is offered as a suggestive outline rather than as a finished plan for milk grading:

GRADE.	ELEMENT OF QUALITY.	DEGREE OF EXCELLENCE.
Special milk	Food value	Fat content as stated on package
	Healthfulness	Medical supervision of health of men and animals, or proper pasteurization
	Cleanliness	Sediment, not more than a trace
	Keeping quality	Excellent

GRADE.	ELEMENT OF QUALITY.	DEGREE OF EXCELLENCE.
Table milk	Food value	Fat content as stated on package
	Healthfulness	Properly pasteurized
	Cleanliness	Sediment, not more than a small amount
	Keeping quality	Good
Cooking milk	Food value	Fat content as stated on package
	Healthfulness	Boiled
	Cleanliness	May not be sufficient for table grade
	Keeping quality	May not be sufficient for table grade

Under the grade of *special milk*, the plan provides for milk of any desired composition to meet any special need, such as baby feeding. The grade of *table milk* will normally include the ordinary city supply. The grade of *cooking milk* is designed for milk not sufficiently fresh or not carefully enough handled to be suitable for the table grade. In order to assist in protecting the consumer from unwittingly purchasing cooking milk instead of table milk, it is suggested that cooking milk be boiled. Such treatment will adequately protect healthfulness, and while making this grade of milk readily recognizable, will not injure it for the use for which it is designed.

RESPONSIBILITY OF THE PRODUCER FOR MILK QUALITY.

If the foregoing analysis of milk quality is correct, the producer sustains relations to each of the elements of milk quality somewhat different from what has been ordinarily supposed. Contrary to current belief, he is unable to control food value to any appreciable extent by his method of feeding the cow. The forces of heredity have determined what shall be the composition of the milk of a given animal, and except for slight seasonal variations or local disturbances a cow persists in giving milk of essentially a fixed composition. On the other hand, by selecting his animals he is able to produce milk of widely different food value, but at a correspondingly different cost of production. Manifestly, it must be expected that he will produce a milk having a food value which will give him the widest margin of profit.

In the matter of healthfulness, the producer has the responsibility of protecting, in so far as he is able, the milk supply from contamination by disease-producing organisms derived either from cows or people. His recognized inability satisfactorily to protect milk in this way calls for the added protection of medical supervision of the health of the cows and men, or of pasteurization; but medical inspection and pasteurization are, manifestly, not the producer's problem.

The element of cleanliness is largely in the control of the producer. Under present economic conditions, he is producing a milk with a very high degree of cleanliness, and if any additional stress is laid upon this point he will undoubtedly produce milk which is uniformly very clean.

The element of keeping quality is the one which presents the greatest practical difficulty, because here the responsibility is much divided. Definite information regarding many details is still lacking, but the present stage of knowledge suggests that the most common factor contributing to poor keeping quality is the condition of the milk cans which are supposed to be properly treated at the milk plants. Where milking machines are used, they are very frequently a large contributing factor to the short keeping quality of the milk.

The adoption by the producer of the uniform practice of rinsing his milk utensils with scalding hot water shortly before they are used, would contribute very much to the keeping quality of the milk. In practice each utensil coming into contact with the milk adds to its germ content and decreases its keeping quality. A reduction of the number of such utensils to the minimum is very desirable.

Under ordinary conditions the udder of the cow contributes but a small number of germs and these have little effect upon the milk. Occasionally, however, cows or even herds are found where the udder content is high and the effect upon the keeping quality of the milk pronounced. Further information is necessary before the true significance of this factor can be accurately estimated.

RESPONSIBILITY OF THE DISTRIBUTOR.

The responsibility of the distributor in the matter of food value concerns itself essentially with conserving the food value of the milk as furnished him by the producer. Where economic conditions permit, he is able to stimulate the production of milk with a higher food value by paying a differential price.

For the healthfulness of milk a heavy responsibility lies upon the distributor, particularly when he is charged with its pasteurization as a final safeguard to the consumer. As a possible source of disease-producing germs, human beings are more dangerous than cows, and a medical supervision of the employees of the milk plant is desirable. This is particularly important in the case of milk pasteurized in bulk, since this process gives no protection from the later contamination, which is a more or less remote possibility during the cooling and bottling processes.

The milk as it comes from the producer usually is and always should be clean. The problem of the distributor is to preserve this cleanliness.

The keeping quality of milk is more largely within the control of the distributor than is usually supposed. He is frequently responsible for the washing and steaming of the milk cans. Where this steaming is done in a perfunctory manner, particularly where tight-fitting covers are applied to wet cans in warm weather, these cans become one of the most important factors in reducing the keeping quality of the milk. Where a proper washing of the cans is followed by a thoro steaming and the cans are carefully dried before being covered, they will have little objectionable effect upon the milk. The large germ content added to milk by utensils within the distributor's plant is frequently an important factor in impairing its keeping quality. The milk coolers and the bottling machines require special watching in this connection, not only because they frequently add large numbers of germs, but especially because they add them *after the milk has been pasteurized.*

The intelligent application of steam to all of the utensils should be a routine procedure, and the flushing out of all utensils with scalding water shortly before using them is a valuable additional precaution.

RESPONSIBILITY OF THE CONSUMER.

The food value of the milk furnished the consumer will depend primarily upon what the consumer desires and is willing to pay for. A considerable proportion of the consumers are desirous of obtaining a milk carrying 4 per ct. of fat or more, and where the milk has been sold regardless of food value they have striven to find the richest milk available at a given price. If each bottle of milk carried a statement of its fat content, the responsibility would then be upon the consumer to recognize and pay for increased food value.

The consumer thru his agents, the health officials, must determine how the healthfulness of the food supply shall be safeguarded. The dangers which naturally surround milk production and handling are such that if the milk supply is to be safe it must be protected either by a medical supervision of the health of the cows and the men or by proper pasteurization, or better, by a combination of both means of protection.

The cleanliness of milk, as it is now delivered to the consumer is in general very satisfactory, but continued emphasis is needed to insure that it shall be constantly maintained at a high level.

The keeping quality of milk is constantly receiving the attention of the consumer, since there is no other shortcoming of milk which is more quickly impressed upon him. The delivery to the consumer of old, stale milk, on the verge of souring, is quite as much a fraud as the delivery of milk deficient in food value, healthfulness, or cleanliness. The consumer is constantly insisting on improvement in keeping quality, and his desires will be met as rapidly as the producer and the distributor find economical means of insuring this improvement.

Since the keeping quality of milk after delivery is dependent primarily upon the temperature at which the milk is held, the responsibility rests upon the consumer to hold the milk at a reasonably low temperature after it is delivered to him. Too frequently little regard is given to this matter by the consumer, and much of the criticism directed against the keeping quality of milk is accordingly unjust.

PREFACE.

The present bulletin will appeal to the average reader as a technical discussion of analytical methods of no immediate interest to him. For that reason this preface is written.

During the past twenty to twenty-five years much has been written in a popular way about the number of bacteria in milk until the general public has come to have the feeling that the average glass of milk is to be looked upon with suspicion. Because of the difficulties involved in making a simple explanation of the methods used in making counts of objects as tiny and as numerous as bacteria, it has become a general custom among dairy bacteriologists to speak of the counts which have been made as counts of the number of bacteria present in milk. At the same time it has been recognized by these men that counts made by the methods in use probably represented counts of "groups" of bacteria rather than counts of single bacteria. Occasionally someone has raised his voice in protest against the general custom of referring to the counts as counts of bacteria; but the protest has been just as frequently ignored.

The development of the new microscopic technique described in a bulletin issued by this Station in 1914 has now made it possible to make reasonably accurate counts of the individual bacteria in milk. The result has been to show that the number of bacteria in milk is much greater than the counts commonly given would indicate. At the same time it has been definitely shown that the chief reason why counts made by the generally used plate method are too small is because they are really counts of masses of bacteria rather than counts of individual bacteria.

It will be a surprise to those who have not realized the probability that this is the case to learn that milk commonly stated to contain 10,000 bacteria per c. c. rarely has less than 15,000 bacteria and may have 250,000 or even more per c. c. Similar conditions hold in regard to other figures that are commonly given.

The confusion which is sure to arise because of the unfortunate situation caused by the previous inaccurate statements can now only be met by care on the part of each person to avoid such inaccuracies in the future.

For the sake of those who may be inclined to look with still greater suspicion on milk when they learn that it contains many more bacteria than they had supposed, it should be stated that recent work in agricultural bacteriology has emphasized the fact that harmful bacteria are the exception rather than the rule. Both sour milk drinks and cheese contain many more bacteria than are present in milk and yet they are known to be healthful foods. The human intestine is known to harbor countless myriads of these tiny organisms in all cases; and yet their presence in the body causes no injury. In fact, such experimental evidence as is available indicates that their presence is an actual benefit. It is only when disease-causing organisms are present that troubles follow.

For the sake of those who, because of selfish or other reasons, would like to conclude that bacteria counts are valueless because they are subject to gross errors, a simple comparison will suffice. In looking at a distant hillside it is not possible to count the trees thereon. Nevertheless it is ordinarily quite possible to form an accurate judgment as to the area covered by woods, by wooded pasture land, and by meadows. Such a judgment might have just as great value for the purpose in hand as would one based upon an accurate count of the trees per acre of land. Bacteria counts of market milk and cream have their value regardless of the difficulties which prevent them from being absolutely accurate. The studies which have been made at this Station and elsewhere upon the sources of bacteria in milk have made it increasingly plain that the correlation between the number of bacteria in milk, and the presence or absence of disease-causing organisms, or of the presence or absence of dirt, dust and filth, is so slight that bacteria counts have little significance with regard to either of these conditions. There remains however a close correlation between the number of bacteria and the state of freshness of the milk or its keeping quality. Milk containing few bacteria ordinarily has a much higher keeping quality than milk containing many bacteria, and the housewife who supposes that she is buying fresh milk which will not sour within a reasonable time should be protected by an efficient inspection service against the milkman who sells her a quart of milk which is nearly sour.

The universal need of inspection in order to control the quality of milk makes studies concerned with improvements or simplifications of the methods of counting bacteria in milk of general interest.

Robert S. Breed.

THE NUMBER OF BACTERIA IN MILK.*

JAMES D. BREW AND W. D. DOTTERRER.

SUMMARY.

1. Comparative counts of the bacteria in 643 samples of unpasteurized market milk were made by the direct microscopic method and the plate method. The counts under the microscope were made in two ways, namely, counts of individual bacteria and counts of " groups " of bacteria.

2. These counts have been arranged in three classes: First, 175 samples (27 per ct.) in which the plate counts were higher than the individual counts. Second, 123 samples (19 per ct.) in which the plate counts were less than the " group " count. Third, 345 samples (54 per ct.) in which the plate counts were greater than the " group " counts and less than the individual counts. The last class of counts are of the type which would be expected in case there were no gross errors in any of the counts.

3. Nearly all of the first class occurred among milk samples containing few bacteria. It seems probable that, because of the small quantity of milk examined under the microscope, bacteria were overlooked. Some of these discrepancies seem to have been caused by the presence of colonies on the plates which developed from unrecognized contaminations.

4. The low plate counts of the second class are due, either to the presence of living bacteria which do not grow on agar, or to the presence of dead bacteria in a stainable condition. Apparently the presence of the former is a more important source of error than the presence of the latter.

5. Generally speaking, the " groups " of bacteria appear to be of small average size in milks containing very few bacteria and to increase in average size as the number of bacteria increase, reaching a maximum average size of 12 to 14 individuals in milk containing less than 1,000,000 bacteria per c. c. As the number of bacteria becomes greater than this the average size of the groups diminishes. Apparently this decrease in the average size of the " groups " in

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* Reprint of Bulletin No. 439, November, 1917.

milk containing large numbers of bacteria is due to the increasing predominance of the lactic acid bacteria.

6. The clumps of bacteria vary greatly in compactness and therefore vary greatly in the amount to which they break apart into smaller clumps in preparing dilutions for the plate method. This causes an error in plate counts which cannot be measured directly. Indirect data indicate that the average clump of bacteria in market milk breaks apart 2.6 times in preparing dilution waters. This varies greatly in individual cases, however, as in 24 instances out of 345 the clumps broke into 7 or more " groups " each. In one instance the resulting number of " groups " was 16.5 times the original number.

7. Because of the serious errors introduced into the plate counts by the presence of clumps of bacteria, as well as by the presence of living bacteria which do not grow on agar, popular ideas as to the number of bacteria in milk, based as they are on plate counts, are incorrect. The actual number of bacteria in milk is usually greatly in excess of the figure obtained by the plating method.

INTRODUCTION.

Quantitative determinations of the germ life in milk, as a means of ascertaining and controlling its wholesomeness as a food, have been quite generally used in the United States. The judging of the quality of milk according to bacterial content has necessitated the establishment of numerical standards which were to be used as guides in classifying it into grades. The increasing use of such standards makes it very important to the commercial interests involved as well as to the officials charged with the enforcement of regulations governing the control of milk supplies, that definite information be secured to determine the accuracy of the analytical methods used.

Bacteriologists have, from the beginning, been aware of the difficulties involved in making accurate bacterial counts and of the wide fluctuations possible in duplicate counts. Many attempts have been made to measure the amount of these errors but they have not proved successful because of the lack of an absolute standard of comparison. The recent improvements in the technique of the direct microscopic method of counting bacteria in milk permit comparisons to be made between counts thus obtained and counts made by the officially recognized agar-plate method. Results secured in this way permit more definite conclusions concerning the accuracy of bacterial counts than have hitherto been possible.

PREVIOUS WORK.

Much valuable work has been done by way of investigating the relationship between such factors as temperature, food requirements, chemical reaction, dilution, etc., and the number of colonies that grow on agar plates. This work, together with the reports drawn up by the Committees on Standard Methods of Analysis appointed by the American Public Health Association,[1] have tended to make the methods used in plating milk samples more uniform. In spite of this, it is well known that there still exists great difficulty in getting uniform plate counts from the same sample of milk when the analyses are made in two different laboratories, or even when the analyses are made by two different workers in the same laboratory.

Probably the most extensive attempt which has been made to so standardize the plate method for making counts of bacteria in milk as to secure uniform results from different laboratories was carried out under the direction of Dr. H. W. Conn [2] working in conjunction with four New York City laboratories which were accustomed to doing routine work. The aim was, first, to determine how great the variation in count would be when duplicate milk samples were plated in each of these laboratories; then, to determine in what way the technique used by each differed. After making as careful a standardization of the methods as found practicable, claim is made that, in subsequent trials, more accurate counts were secured because there was a better agreement in the counts obtained by the different laboratories.

An objection which might be made to this claim is that it is impossible to tell when a count of bacteria is accurate. This is because it is exceedingly difficult to count accurately large numbers of any object, and the smaller the objects are, the nearer the task of counting approaches impossibility. A large office force is required to count the several million dollars of gold, silver, and bank notes stored in the United States Treasury vaults. But money is large enough to be handled separately and the different denominations lend themselves readily to systematization. Since this is not true with reference to objects so numerous and tiny as bacteria, actual counting is out of the question. Therefore the best that can be done is to make estimates of the numbers present; and the exact number of bacteria not being known, the accuracy of any " count " or method of making estimates cannot be determined absolutely, because there is no standard with which to compare.

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[1] Report. *Amer. Jour. Pub. Hyg.*, **20** (**N. S. 6**):315–345. 1910. Also Report *Amer. Jour. Pub. Health*, **6**:1315–1325. 1916.

[2] Conn, H. W. Standards for determining the purity of milk. U. S. Pub. Health Serv., *Pub. Health Reports*, **30**:2349–2395. 1915.

Even if a perfect standardization of the plate method results in securing ideally uniform counts in duplicate samples of milk, this does not prove the counts to be accurate. There may be a uniform error common to all the samples. Such an error, for example, might be caused by the existence of clumps of bacteria in the milk.

Also, uniform counts from duplicate samples do not prove that the same relative error exists thruout a series of counts made from different samples of milk. The count on one sample may be affected by one type of error and the count on a second by another type. For instance, one sample may contain a large number of bacteria not adapted to growing on agar while another sample may contain approximately the same number of bacteria all of which are capable of growing on agar. Tho the percentage error may be uniform in duplicate samples of milk it can be readily understood how the percentage error can be widely divergent in different samples.

Altho the number of bacteria in milk can be stated as estimates only, it is possible to learn something of the probable accuracy of these estimates when made by two or more different methods. The direct microscopic method as devised by Breed,[3] and the plate method used simultaneously on the same samples of milk furnish a comparatively simple means for making such comparisons.

Such a study has been carried on at this Station in two ways. First, coöperating with the Department of Dairy Industry at Cornell University, comparative analyses have been made by several men on a small number of duplicate milk samples. A preliminary account of this work is reported in the *Journal of Dairy Science.*[4] Second, comparative studies have been made at this Station on miscellaneous market milk samples. The first report of the latter work was a preliminary one submitted in 1914.[5] A second bulletin [6] described the technique after it had been further developed in routine work. The present bulletin contains a report on 643 comparative counts made by both the plate and microscopic methods.

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[3] Breed, R. S. The determination of the number of bacteria in milk by direct microscopical examination. *Centbl. Bakt.*, Abth. II, 30:337–340. 1911.

PLAN OF WORK.

The milk selected for this investigation was fresh, unpasteurized market milk from the Geneva city milk supply. Each sample was taken by means of a sterile aluminum pipette which was long enough to reach to the bottom of a 40-quart can. The night milk was always older than 12 hours and usually less than 16 hours old, while the morning milk was rarely older than four hours. The samples were packed in ice as soon as taken. It required approximately four hours to take the samples, bring them to the laboratory and to plate them.

Plating method.—The medium used for plating the first 491 samples was made from meat extract, peptone and agar according to the following formula:

Liebig's beef extract	5 grams.
Witte's peptone	10 grams.
Agar (shreds)	15 grams.
Distilled water	1000 cubic centimeters.

The acidity of each lot of medium was determined by direct titration and was found to be very constant. The percentage acidity to phenolphthalein ranged from 0.8 to 1.1 and no attempt was made to adjust the reaction. The H-ion concentration of the medium made according to this formula, as determined by the colorometric method, has been found to be between $P_H = 7.0$ and $P_H \doteq 6.5$.

Ten grams of lactose per liter were added to the medium used for plating the remaining 152 samples.

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[4] Breed, R. S., and Stocking, W. A., Jr. A preliminary report on a series of co-operative bacterial analyses of milk. *Jour. Dairy Sci.*, 1:19–35. 1917.

[5] Brew, J. D. A comparison of the microscopical method and the plate method of counting bacteria in milk. N. Y. Agr. Exp. Sta. Bul. 373, pp. 1–38, 1 pl., 2 figs. 1914.

[6] Breed, R. S., and Brew, J. D. Counting bacteria by means of the microscope. N. Y. Agr. Exp. Sta. Tech. Bul. 49, pp. 1–31, 5 figs. 1916.

Method of plating.—Three dilutions (1/100, 1/1000, 1/10,000) were used in all cases and each dilution was plated in triplicate. Each sample of milk was shaken vigorously 50 times and each diluted sample 25 times. Care was taken to keep the interval of time elapsing between the making of the dilutions and the pouring of the plates less than 20 minutes.

Incubation.—All plates were incubated at 21° C. for five days and then counted with the aid of a magnifying lens. They were then incubated two additional days at 37° C. and recounted. The plate counts herein reported are the higher of the two, but since there was generally a slight increase in the number of colonies after the additional incubation at 37° nearly all of the counts reported were those obtained after this incubation.

Counting plates.—In selecting the plates for counting, only those were chosen which had more than 20 and less than 400 colonies.[7] There were necessarily a few exceptions which have been noted in the tables. In only 10 cases were all plates so crowded as to make it necessary to estimate the number of colonies on each. Triplicate platings in three dilutions made it possible to secure a final count which was an average usually from three or more plates. All of those cases in which it was impossible to count more than one plate have been noted in the tables.

Microscopic method.—As soon as the dilutions for plating were completed, preparations were made for microscopic examination according to the procedure described in Technical Bulletin No. 49.[8]

The microscope was adjusted so as to use a 300,000 factor for computing the number of bacteria found in the microscopic fields in terms of bacteria per cubic centimeter. In order to obtain this factor with the microscope at hand a 5x ocular, and a 1.9 mm. oil immersion objective were used, and the draw tube was placed at 170. This gave a microscopic field .205 millimeters in diameter.

Counting.—In order to secure as great uniformity as possible in microscopic counts, certain definite rules were observed which may be stated as follows:

1. The bacteria in 100 microscopic fields were counted in all cases where they were few in number. With a 300,000 factor this

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[7] Breed, R. S., and Dotterrer, W. D. The number of colonies allowable on satisfactory agar plates. N. Y. Agr. Exp. Sta. Tech. Bul. 53, pp. 1–11. 1916.

[8] See footnote 6.

means that $\frac{1}{3000}$ cubic centimeter of milk was actually viewed under the microscope. If the bacteria were fairly numerous the number of fields counted was reduced to not less than 30, in which case $\frac{1}{10,000}$ cubic centimeter of milk was viewed. If the bacteria were very numerous the number of fields counted was still further reduced to 10 or even 5 fields which were ruled off with an ocular micrometer. The ruled area on the micrometer in this case was of such a size and the microscope so adjusted that the factor necessary to transform the results to the cubic centimeter basis was 600,000. When only 5 fields were counted $\frac{1}{120,000}$ cubic centimeter of milk was viewed.

2. The microscopic counts were made in two ways, a count of the "groups" per cubic centimeter and a count of the individual bacteria per cubic centimeter. Isolated organisms or clumps of two or more organisms were recorded as a "group." The word "group" is used in this sense thruout the present publication.

3. Whenever a clump was too large or too dense to count accurately an estimate of the number of individuals was made.

4. An organism showing clear indication of division was counted as two individuals.

5. Any stained object about which there was doubt as to whether or not it was a bacterium was not counted.

Comparative counts as made by the two methods.—In the discussion of the results in the following tables it is necessary to keep in mind what the count as obtained by each method actually represents.

The plate count represents the number of colonies on agar of those bacteria alone which are adapted to growth under the conditions offered by this technique. This being true, then "groups" made up entirely of dead organisms or of organisms incapable of growth on the agar plate are not included in the count. The colonies on the plate may originate from a single organism or from a clump containing varying numbers of individuals. Since the clumps originally present in the milk break apart to some extent in preparing dilutions, the plate count does not represent either the original number of "groups" of bacteria present in the milk, or the total number of bacteria; but a figure intermediate between the two.

A count by the direct microscopic method may be made so as to represent either of two things; first the total number of individual bacteria, and, second, the number of "groups" of bacteria originally present in the milk. This latter count is usually more nearly the same as the plate count than is the individual count but differs from it in that it represents the " groups " as they actually occur in the milk, not as broken apart by shaking in the dilution waters. In both kinds of microscopic counts, all organisms (including such dead organisms as have not lost their staining power, and the live ones which do not grow on agar) are seen and counted.

The relationship between counts made by the two methods is complicated by four important and uncontrollable sources of error which may be stated as follows:

1. Errors due to the occurrence of bacteria in clumps.
2. Errors due to variation in breaking apart of clumps.
3. Errors due to the presence of live bacteria which do not grow.
4. The microscopic count includes such dead bacteria as are present in stainable condition and may differ greatly from the plate count.

The first source of error may be measured directly by microscopic examination, while the second may be measured indirectly by comparing the " group " count as made by microscopic examination with the plate count. As yet no method has been found whereby the errors due to the third and fourth causes can be measured either directly or indirectly.

These uncontrollable sources of error make the actual errors in count large ones and make it difficult to measure the amount of the error even where comparative counts by two different methods are available. In spite of this fact, comparative studies contribute much valuable information concerning the accuracy of " bacterial estimates " and the causes of the variations therein.

DATA.

Tables I and II contain the counts made from 643 samples of raw market milk.* Table I contains the counts made from 491 samples which were plated on plain agar and Table II contains the counts from 152 samples which were plated on lactose agar.

According to the experience of other investigators the addition of lactose to the agar medium usually results in a higher plate count.

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* Three hundred and seventy-five of the samples were plated by the junior author. The remainder of the analytical work was done by the senior author.

This being true it was expected that the addition of the lactose to the agar used for the last 152 samples would result in higher plate counts. The consequence of this would have been that the average ratio between the plate counts and the " group " counts would be greater than when plain agar was used; and the average ratio between the plate counts and the individual counts would be less. Since however there was no marked indication of a beneficial result following the addition of the lactose, the two tables have been discussed as one.

The 643 counts are arranged in each table according to the number of individual bacteria, beginning with the lowest count. The second column contains the microscopic counts made of the individual bacteria, the third column contains the microscopic count of " groups " of bacteria, while the fourth column shows the average size of the " groups." The fifth column contains the count made by the plate method and the sixth column the ratio between the " group " and the plate count. Theoretically this ratio indicates the extent to which the " groups " of bacteria break apart in the plating process.

In discussing these tables, it is important to keep in mind the relationship which would be expected to hold between counts obtained by the two methods. As previously stated the counts made under the microscope show the number of " groups " per cubic centimeter and the number of individual bacteria per cubic centimeter. Since the clumps of bacteria originally present in milk tend to break apart during the process of dilution for the plate method, then the plate count would naturally be expected to be larger than the " group " count and always less than the total number of individuals. It seems, therefore, fair to assume that all counts which satisfy these conditions may be considered as showing the normal theoretical relationship. Counts which do not satisfy these conditions are influenced by some source of error sufficiently to disturb this normal relationship.

The counts obtained from the 643 samples can be arranged in three classes. The first includes the 175 samples (27 per ct.) which gave plate counts greater than the individual count. These counts are printed in ordinary type. The second includes the 123 samples (19 per ct.) which gave plate counts less than the " group " count. These counts are printed in italics. The third includes the 345 samples (54 per ct.) which show the normal theoretical relationship mentioned above. These latter counts are printed in bold faced type.

An examination of the tables will show at once that the three classes of counts are not evenly distributed thruout the whole series of counts. The largest number of samples in which the plate counts were greater than the individual counts (class I) are found in milk containing few bacteria. Only 32 of a total of 175 counts in this class occur among those samples showing more than 30,000 individual bacteria per cubic centimeter. The 123 samples which gave plate counts less than the "group" count (class II) occur quite evenly distributed thruout the whole range of counts. Counts which show the normal theoretical relationship (class III) are not common among the samples showing less than 30,000 individual bacteria per cubic centimeter, only 28 of the 200 samples containing less than 30,000 bacteria being of this type.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD.

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
1	**Less than 3,000 per c. c.**		....	1,900	....	Under 20 colonies per plate.
2	**Less than 3,000 per c. c.**		....	2,130	....	
3	**Less than 3,000 per c. c.**		....	2,230	....	
4	**Less than 3,000 per c. c.**		....	2,850	....	
5	**Less than 3,000 per c. c.**		....	2,870	....	
6	Less than 3,000 per c. c.		....	3,030	....	
7	Less than 3,000 per c. c.		....	3,400	....	Only one plate counted.
8	Less than 3,000 per c. c.		....	3,550	....	
9	Less than 3,000 per c. c.		....	3,570	....	
10	Less than 3,000 per c. c.		....	4,250	....	
11	Less than 3,000 per c. c.		....	4,600	....	
12	Less than 3,000 per c. c.		....	5,270	....	
13	Less than 3,000 per c. c.		....	5,500	....	
14	Less than 3,000 per c. c.		....	5,600	....	
15	Less than 3,000 per c. c.		....	5,930	....	
16	Less than 3,000 per c. c.		....	6,100	....	
17	Less than 3,000 per c. c.		....	6,120	....	
18	Less than 3,000 per c. c.		....	6,330	....	
19	Less than 3,000 per c. c.		....	6,420	....	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
20	Less than 3,000 per c. c.		....	6,980	....	
21	Less than 3,000 per c. c.		....	7,330	....	
22	Less than 3,000 per c. c.		....	7,370	....	
23	Less than 3,000 per c. c.		....	7,500	....	
24	Less than 3,000 per c. c.		....	7,730	....	
25	Less than 3,000 per c. c.		....	7,850	....	
26	Less than 3,000 per c. c.		....	8,140	....	
27	Less than 3,000 per c. c.		....	8,400	....	
28	Less than 3,000 per c. c.		....	8,550	....	
29	Less than 3,000 per c. c.		....	8,730	....	
30	Less than 3,000 per c. c.		....	8,930	....	
31	Less than 3,000 per c. c.		....	9,100	....	
32	Less than 3,000 per c. c.		....	9,200	....	
33	Less than 3,000 per c. c.		....	9,900	....	
34	Less than 3,000 per c. c.		....	10,000	....	
35	Less than 3,000 per c. c.		....	11,000	....	
36	Less than 3,000 per c. c.		....	11,000	....	
37	Less than 3,000 per c. c.		....	12,000	....	
38	Less than 3,000 per c. c.		....	12,000	....	
39	Less than 3,000 per c. c.		....	14,000	....	
40	Less than 3,000 per c. c.		....	14,000	....	
41	Less than 3,000 per c. c.		....	15,000	....	
42	Less than 3,000 per c. c.		....	16,000	....	
43	Less than 3,000 per c. c.		....	17,000	....	
44	Less than 3,000 per c. c.		....	17,000	....	
45	Less than 3,000 per c. c.		....	19,000	....	
46	Less than 3,000 per c. c.		....	19,000	....	
47	Less than 3,000 per c. c.		....	25,000	....	
48	Less than 3,000 per c. c.		....	32,000	....	
49	Less than 3,000 per c. c.		....	33,000	....	
50	Less than 3,000 per c. c.		....	38,000	....	
51	Less than 3,000 per c. c.		....	38,000	....	
52	Less than 3,000 per c. c.		....	43,000	....	Crowded plates.
53	3,000	3,000	1.0	2,130	....	
54	3,000	3,000	1.0	2,530	....	
55	3,000	3,000	1.0	4,500	....	
56	3,000	3,000	1.0	4,930	....	
57	3,000	3,000	1.0	5,100	....	
58	3,000	3,000	1.0	5,500	....	
59	3,000	3,000	1.0	5,800	....	Only one plate counted.
60	3,000	3,000	1.0	6,200	....	Only one plate counted.
61	3,000	3,000	1.0	7,730	....	
62	3,000	3,000	1.0	8,200	....	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
63	3,000	3,000	1.0	8,300	....	Only one plate counted.
64	3,000	3,000	1.0	9,880	....	
65	3,000	3,000	1.0	10,000	....	
66	3,000	3,000	1.0	11,000	....	
67	3,000	3,000	1.0	14,000	....	
68	3,000	3,000	1.0	15,000	....	Only one plate counted.
69	3,000	3,000	1.0	20,000	....	Plate count at 21°=5,230.
70	3,000	3,000	1.0	146,000	....	Recount of smear, 33,000 "groups" and 154,000 individuals.
71	*6,000*	*3,000*	*2.0*	*1,830*	....	Less than 20 colonies per plate.
72	*6,000*	*3,000*	*2.0*	*2,150*	....	
73	6,000	3,000	2.0	4,300	1.4	Only one plate counted.
74	6,000	3,000	2.0	6,130	....	
75	6,000	3,000	2.0	6,570	....	
76	6,000	3,000	2.0	7,240	....	
77	6,000	3,000	2.0	8,230	....	
78	6,000	3,000	2.0	11,000	....	
79	6,000	3,000	2.0	11,000	....	
80	6,000	3,000	2.0	15,000	....	
81	6,000	3,000	2.0	15,000	....	
82	6,000	3,000	2.0	17,000	....	
83	6,000	3,000	2.0	19,000	....	
84	6,000	3,000	2.0	24,000	....	
85	6,000	3,000	2.0	25,000	....	
86	6,000	3,000	2.0	43,000	....	
87	6,000	3,000	2.0	47,000	....	
88	6,000	3,000	2.0	60,000	....	
89	6,000	3,000	2.0	61,000	....	
90	6,000	3,000	2.0	83,000	....	
91	6,000	3,000	2.0	126,000	....	
92	*6,000*	*6,000*	*1.0*	*1,570*	....	Less than 20 colonies per plate.
93	*6,000*	*6,000*	*1.0*	*2,900*	....	
94	*6,000*	*6,000*	*1.0*	*4,820*	....	
95	*6,000*	*6,000*	*1.0*	*5,370*	....	
96	6,000	6,000	1.0	7,930	....	
97	6,000	6,000	1.0	8,750	....	
98	6,000	6,000	1.0	14,000	....	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
99	6,000	6,000	1.0	15,000	....	Less than 20 colonies per plate.
100	9,000	3,000	3.0	14,000	....	
101	9,000	3,000	3.0	19,000	....	
102	9,000	3,000	3.0	26,000	....	
103	*9,000*	*6,000*	*1.5*	*2,850*	....	
104	*9,000*	*6,000*	*1.5*	*4,530*	....	
105	9,000	6,000	1.5	20,000	....	Only one plate counted.
106	9,000	6,000	1.5	22,000	....	
107	9,000	6,000	1.5	39,000	....	
108	*9,000*	*9,000*	*1.0*	*4,000*	....	
109	9,000	9,000	1.0	11,000	....	
110	**12,000**	**3,000**	**4.0**	**5,530**	**1.8**	
111	**12,000**	**3,000**	**4.0**	**7,230**	**2.4**	
112	**12,000**	**3,000**	**4.0**	**7,680**	**2.6**	
113	**12,000**	**3,000**	**4.0**	**11,000**	**3.7**	
114	12,000	3,000	4.0	12,000	....	
115	12,000	3,000	4.0	12,000	....	
116	12,000	3,000	4.0	15,000	....	
117	12,000	3,000	4.0	16,000	....	
118	*12,000*	*6,000*	*2.0*	*2,230*	....	
119	*12,000*	*6,000*	*2.0*	*3,030*	....	
120	*12,000*	*9,000*	*1.3*	*3,530*	....	
121	*12,000*	*9,000*	*1.3*	*4,800*	....	
122	*12,000*	*9,000*	*1.3*	*4,900*	....	Only one plate counted.
123	*12,000*	*9,000*	*1.3*	*7,230*	....	
124	*12,000*	*9,000*	*1.3*	*8,500*	....	
125	12,000	9,000	1.3	13,000	....	
126	12,000	9,000	1.3	32,000	....	
127	*12,000*	*12,000*	*1.0*	*8,500*	....	
128	*12,000*	*12,000*	*1.0*	*9,650*	....	
129	*15,000*	*6,000*	*2.5*	*2,930*	....	
130	**15,000**	**6,000**	**2.5**	**6,550**	**1.1**	
131	15,000	6,000	2.5	1,493,000	....	Recount of smears gave similar results.
132	*15,000*	*9,000*	*1.7*	*5,930*	....	
133	*15,000*	*12,000*	*1.3*	*4,100*	....	
134	15,000	12,000	1.3	19,000	....	
135	*15,000*	*15,000*	*1.0*	*10,000*	....	
136	**18,000**	**3,000**	**6.0**	**13,000**	**4.3**	
137	18,000	3,000	6.0	35,000	....	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
138	*18,000*	*6,000*	*3.0*	*3.300*	....	
139	18,000	6,000	3.0	19,000	....	
140	18,000	6,000	3.0	52,000	....	
141	18,000	9,000	2.0	14,000	1.6	
142	18,000	9,000	2.0	15,000	1.7	
143	18,000	9,000	2.0	19,000	....	
144	18,000	9,000	2.0	33,000	....	
145	*18,000*	*12,000*	*1.5*	*5,770*	....	
146	18,000	12,000	1.5	16,000	1.3	
147	18,000	12,000	1.5	24,000	....	
148	18,000	12,000	1.5	102,000	....	
149	*18,000*	*15,000*	*1.2*	*4,870*	....	
150	*18,000*	*15,000*	*1.2*	*5,180*	....	
151	*18,000*	*18,000*	*1.0*	*2,500*	....	
152	18,000	1,500	10.2	8,450	5.6	
153	21,000	6,000	3.5	16,000	2.7	
154	21,000	9,000	2.3	11,000	1.2	
155	21,000	9,000	2.3	61,000	....	
156	21,000	9,000	2.3	67,000	....	
157	21,000	12,000	1.8	13,000	1.1	
158	21,000	9,000	2.3	42,000	....	
159	*24,000*	*3,000*	*8.0*	*1,560*	....	Less than 20 colonies per plate.
160	*24,000*	*3,000*	*8.0*	*2,650*	....	
161	24,000	6,000	4.0	24,000	....	
162	*24,000*	*9,000*	*2.7*	*5,670*	....	
163	27,000	6,000	4.5	14,000	2.3	
164	27,000	9,000	3.0	27,000	....	
165	*27,000*	*12,000*	*2.3*	*8,000*	....	
166	27,000	12,000	2.3	58,000	....	
167	27,000	21,000	1.3	31,000	....	
168	30,000	3,000	10.0	7,460	2.5	
169	30,000	3,000	10.0	14,000	4.7	
170	30,000	6,000	5.0	8,620	1.4	
171	30,000	6,000	5.0	9,200	1.5	
172	30,000	6,000	5.0	12,000	2.0	
173	30,000	9,000	3.3	12,000	1.3	
174	30,000	9,000	3.3	24,000	2.7	
175	30,000	9,000	3.3	33,000	....	
176	30,000	9,000	3.3	126,000	....	
177	30,000	9,000	3.3	148,000	....	
178	30,000	12,000	2.5	25,000	2.1	
179	30,000	18,000	1.7	28,000	1.6	
180	*30,000*	*21,000*	*1.4*	*19,000*	....	
181	32,000	12,000	2.7	15,000	1.3	

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[1] All counts per cubic centimeter of milk.

TABLE I.—COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
182	33,000	3,000	11.0	5,670	1.9	
183	33,000	6,000	5.5	28,000	4.7	
184	33,000	12,000	2.8	13,000	1.1	
185	*36,000*	*6,000*	*6.0*	*1,570*	....	Less than 20 colonies per plate.
186	36,000	6,000	6.0	15,000	2.5	
187	36,000	6,000	6.0	17,000	2.8	
188	36,000	6,000	6.0	47,000	....	
189	36,000	6,000	6.0	54,000	....	
190	36,000	9,000	4.0	14,000	1.6	
191	36,000	15,000	2.4	37,000	....	
192	36,000	21,000	1.7	28,000	1.3	
193	*37,500*	*10,500*	*3.6*	*3,400*	....	
194	39,000	6,000	6.5	8,770	1.5	
195	39,000	12,000	3.3	33,000	2.8	
196	39,000	15,000	2.6	39,000	....	
197	42,000	6,000	7.0	11,000	1.8	
198	42,000	6,000	7.0	25,000	4.2	
199	*42,000*	*12,000*	*3.5*	*1,760*	....	Less than 20 colonies per plate.
200	42,000	12,000	3.5	16,000	1.3	
201	*42,000*	*15,000*	*2.8*	*13,000*	....	Plate count at 21°=6,200.
202	*42,000*	*15,000*	*2.8*	*13,000*	....	
203	42,000	15,000	2.8	75,000	....	Less than 20 colonies per plate.
204	42,000	30,000	1.4	78,000	....	
205	*42,000*	*36,000*	*1.2*	*32,000*	....	
206	45,000	3,000	15.0	8,470	2.8	
207	45,000	3,000	15.0	22,000	7.3	
208	45,000	12,000	3.8	64,000	....	
209	45,000	27,000	1.7	62,000	....	Only one plate count.
210	48,000	3,000	16.0	5,500	1.8	
211	48,000	6,000	8.0	13,000	2.2	
212	48,000	6,000	8.0	45,000	7.5	
213	*48,000*	*9,000*	*5.3*	*3,000*	....	Less than 20 colonies per plate.
214	48,000	9,000	5.3	9,200	1.0	
215	48,000	12,000	4.0	34,000	2.8	
216	48,000	15,000	3.2	20,000	1.3	
217	48,000	27,000	1.8	38,000	1.4	
218	51,000	21,000	2.4	72,000	....	
219	54,000	6,000	9.0	7,530	1.3	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
220	54,000	6,000	9.0	10,000	1.7	Less than 20 colonies on 1 plate only.
221	54,000	6,000	9.0	12,000	2.0	
222	54,000	12,000	4.5	40,000	3.3	
223	*54,000*	*15,000*	*3.6*	*11,000*	....	
224	57,000	27,000	*2.1*	59,000	....	
225	57,000	30,000	1.9	40,000	1.3	Precipitate in smears.
226	60,000	3,000	20.0	8,030	2.7	
227	60,000	6,000	10.0	19,000	3.2	
228	60,000	27,000	2.2	64,000	....	
229	63,000	12,000	5.3	18,000	1.5	
230	63,000	12,000	5.3	73,000	....	
231	63,000	12,000	5.3	85,000	....	
232	63,000	21,000	3.0	59,000	2.8	
233	*66,000*	*6,000*	*11.0*	*3,330*	....	
234	66,000	6,000	11.0	12,000	2.0	
235	66,000	6,000	11.0	14,000	2.3	
236	*66,000*	*51,000*	*1.3*	*39,000*	....	Only one plate counted.
237	*72,000*	*15,000*	*4.8*	*11,000*	....	
238	72,000	15,000	4.8	41,000	2.7	
239	72,000	18,000	4.0	31,000	1.7	
240	72,000	21,000	3.4	22,000	1.1	
241	72,000	27,000	2.7	71,000	2.6	
242	75,000	12,000	6.3	20,000	1.7	
243	78,000	9,000	8.7	15,000	1.7	
244	78,000	18,000	4.3	39,000	2.2	
245	*78,000*	*33,000*	*2.4*	*14,000*	....	
246	*78,000*	*36,000*	*2.2*	*35,000*	....	
247	79,500	13,500	5.9	26,000	1.9	
248	*81,000*	*36,000*	*2.3*	*25,000*	....	
249	84,000	6,000	14.0	9,560	1.6	
250	84,000	9,000	9.3	60,000	6.7	
251	84,000	18,000	4.7	38,000	2.1	
252	*87,000*	*9,000*	*9.7*	*6,870*	....	
253	87,000	9,000	9.7	12,000	1.3	
254	87,000	33,000	2.6	78,000	2.4	
255	87,000	39,000	2.2	143,000	....	
256	90,000	3,000	30.0	11,000	3.7	
257	90,000	9,000	10.0	36,000	4.0	
258	90,000	18,000	5.0	33,000	1.8	
259	90,000	21,000	4.3	61,000	2.9	
260	93,000	21,000	4.4	45,000	2.1	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
261	93,000	27,000	3.4	36,000	1.3	
262	93,000	45,000	2.1	50,000	1.1	Only one plate counted.
263	94,500	7,500	12.6	14,000	1.9	
264	96,000	18,000	5.3	29,000	1.6	
265	99,000	12,000	8.3	16,000	1.3	
266	99,000	12,000	8.3	142,000	....	
267	99,000	18,000	5.5	63,000	3.5	
268	100,000	40,000	2.5	93,000	2.3	
269	100,500	10,500	9.6	33,000	3.1	
270	*102,000*	*6,000*	*17.0*	*3,000*	....	Less than 20 colonies per plate.
271	102,000	27,000	3.8	56,000	2.1	
272	*105,000*	*18,000*	*5.8*	*5,450*	....	
273	105,000	18,000	5.8	26,000	1.4	
274	105,000	39,000	2.7	130,000	....	
275	107,000	42,000	2.5	125,000	....	
276	108,000	15,000	7.2	43,000	2.9	Only one plate counted.
277	*108,000*	*24,000*	*4.5*	*21,000*	....	
278	108,000	30,000	*3.6*	131,000	....	
279	110,000	20,000	5.5	32,000	1.6	
280	111,000	21,000	5.3	28,000	1.3	
281	*114,000*	*21,000*	*5.4*	*2,600*	....	
282	114,000	27,000	4.2	84,000	3.1	
283	117,000	54,000	2.2	110,000	2.0	Less than 20 colonies on one plate only.
284	120,000	9,000	13.3	42,000	4.7	
285	120,000	12,000	10.0	29,000	2.4	
286	123,000	9,000	13.7	11,000	1.2	
287	123,000	21,000	5.9	62,000	3.0	
288	123,000	24,000	5.0	50,000	2.1	Less then 20 colonies per plate.
289	124,500	22,500	5.5	113,000	5.0	
290	126,000	24,000	5.3	64,000	2.7	
291	129,000	12,000	10.8	24,000	2.0	
292	129,000	24,000	5.4	78,000	3.3	
293	132,000	6,000	22.0	151,000	....	
294	132,000	9,000	15.8	187,000	....	
295	135,000	12,000	11.3	17,000	1.4	
296	*135,000*	*30,000*	*4.5*	*19,000*	....	
297	141,000	18,000	7.8	37,000	2.1	
298	141,000	39,000	3.6	90,000	2.3	
299	144,000	9,000	16.0	29,000	3.2	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
300	144,000	69,000	2.1	154,000	....	
301	147,000	54,000	2.7	169,000	....	
302	150,000	15,000	10.0	37,000	2.5	
303	150,000	21,000	7.1	24,000	1.1	
304	150,000	21,000	7.1	48,000	2.3	
305	150,000	27,000	5.6	108,000	4.0	
306	150,000	54,000	2.8	28,000	....	
307	153,000	27,000	5.6	47,000	1.7	
308	156,000	7,500	20.8	9,980	1.3	
309	156,000	30,000	5.2	136,000	4.5	
310	159,000	12,000	13.3	20,000	1.7	
311	159,000	12,000	13.3	24,000	2.0	
312	159,000	39,000	4.1	74,000	1.9	
313	*160,000*	*130,000*	*1.1*	*73,000*	....	
314	*162,000*	*33,000*	*4.9*	*32,000*	....	
315	162,000	27,000	6.0	123,000	4.6	
316	168,000	12,000	14.0	17,000	1.4	
317	*168,000*	*24,000*	*7.0*	*22,000*	....	
318	168,000	27,000	6.2	150,000	5.6	
319	168,000	36,000	4.7	64,000	1.8	
320	170,000	50,000	3.4	184,000	....	
321	171,000	6,000	28.5	99,000	16.5	
322	174,000	18,000	9.7	29,000	1.6	
323	174,000	90,000	1.9	106,000	1.2	
324	177,000	6,000	28.2	32,000	5.3	
325	177,000	45,000	3.9	74,000	1.6	
326	180,000	30,000	6.0	38,000	1.3	
327	*180,000*	*80,000*	*2.3*	*62,000*	....	Only one plate counted.
328	180,000	87,000	2.1	126,000	1.4	
329	180,000	3,000	60.0	4,700	1.7	
330	183,000	27,000	6.8	75,000	2.8	
331	192,000	66,000	2.9	116,000	1.8	
332	192,000	108,000	1.8	113,000	1.0	
333	198,000	3,000	66.0	8,500	2.8	
334	200,000	30,000	6.7	54,000	1.8	
335	204,000	60,000	3.4	80,000	1.3	
336	207,000	9,000	23.0	65,000	7.2	
337	210,000	40,000	5.3	58,000	1.5	Only one plate counted.
338	213,000	48,000	4.4	113,000	2.4	
339	219,000	18,000	12.2	37,000	2.1	
340	225,000	24,000	9.4	116,000	4.8	
341	228,000	9,000	25.3	11,000	1.2	
342	*234,000*	*15,000*	*15.6*	*11,000*	....	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
343	234,000	15,000	15.6	67,000	4.5	
344	240,000	15,000	16.0	247,000	....	
345	*240,000*	*18,000*	*13.3*	*18,000*	....	Plate count at 21° = 5,480.
346	246,000	18,000	13.7	24,000	1.3	
347	*246,000*	*21,000*	*11.7*	*14,000*	....	
348	*249,000*	*3,000*	*83.0*	*2,070*	....	
349	252,000	75,000	3.4	111,000	1.5	
350	260,000	80,000	2.3	164,000	2.1	
351	261,000	30,000	8.4	58,000	1.9	
352	270,000	9,000	30.0	59,000	6.6	
353	270,000	30,000	9.0	84,000	2.8	
354	270,000	33,000	8.2	122,000	3.7	
355	270,000	70,000	3.9	132,000	1.7	
356	270,000	80,000	3.5	101,000	1.3	
357	282,000	18,000	15.7	1,477,000	....	Recount of smears same.
358	285,000	30,000	9.5	74,000	2.5	
359	*290,000*	*150,000*	*1.9*	*77,000*	....	
360	*294,000*	*15,000*	*19.6*	*6,400*	....	Only one plate counted.
361	297,000	27,000	11.0	67,000	2.5	
362	297,000	36,000	8.3	83,000	2.4	
363	300,000	36,000	8.3	97,000	2.7	
364	312,000	48,000	6.5	65,000	1.4	
365	315,000	24,000	13.1	53,000	2.3	Over 500 colonies per plate.
366	315,000	33,000	9.5	124,000	3.8	
367	320,000	60,000	5.3	65,000	1.1	
368	321,000	36,000	8.9	96,000	2.7	
369	339,000	99,000	3.4	139,000	1.1	
370	*342,000*	*45,000*	*7.6*	*18,000*	....	
371	*348,000*	*120,000*	*2.9*	*91,000*	....	
372	*351,000*	*51,000*	*6.9*	*27,000*	....	
373	351,000	84,000	4.2	235,000	2.8	
374	355,000	60,000	5.9	68,000	1.1	
375	364,000	46,500	7.8	221,000	4.7	
376	372,000	18,000	20.7	90,000	5.0	
377	*381,000*	*33,000*	*11.6*	*23,000*	....	
378	384,000	48,000	8.0	159,000	3.3	
379	*390,000*	*140,000*	*2.8*	*137,000*	....	
380	400,000	40,000	10.0	62,000	1.6	
381	400,000	75,000	5.3	88,000	1.2	
382	408,000	21,000	19.4	29,000	1.4	
383	408,000	30,000	13.6	38,000	1.3	

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[1] All counts per cubic centimeter of milk.

Table I.— Colonies[1] on Plain Agar, and Bacteria[1] Found by the Direct Microscopic Method (*continued*).

Sample No.	Microscopic Method.			Number colonies on agar plates.	Average breaking up of "groups."	Notes
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
384	408,000	60,000	6.8	180,000	3.0	Less than 20 colonies on 1 plate only.
385	408,000	102,000	4.0	119,000	1.2	
386	420,000	36,000	11.7	150,000	4.2	Less than 20 colonies per plate.
387	420,000	120,000	3.5	223,000	1.9	
388	444,000	30,000	14.8	127,000	4.2	
389	*455,000*	*45,000*	*11.1*	*33,000*	....	
390	480,000	78,000	6.2	152,000	1.9	
391	498,000	75,000	6.6	128,000	1.7	
392	500,000	50,000	10.0	255,000	5.1	
393	537,000	72,000	7.5	109,000	1.5	
394	540,000	60,000	9.0	70,000	1.2	
395	540,000	100,000	5.4	145,000	1.5	
396	558,000	27,000	20.7	243,000	9.0	
397	570,000	3,000	190.0	11,000	3.7	
398	*582,000*	*42,000*	*13.9*	*36,000*	....	
399	588,000	60,000	9.5	134,000	2.2	
400	600,000	102,000	5.9	176,000	1.7	
401	612,000	39,000	15.7	71,000	1.8	
402	633,000	42,000	15.1	151,000	3.6	Plate count at 21° = 103,000.
403	*636,000*	*66,000*	*9.6*	*55,000*	....	
404	660,000	130,000	5.1	322,000	2.5	
405	*684,000*	*114,000*	*6.0*	*107,000*	....	
406	690,000	24,000	28.8	55,000	2.3	
407	696,000	33,000	21.9	51,000	1.6	
408	*700,000*	*90,000*	*7.8*	*21,000*	....	
409	714,000	28,500	25.1	119,000	4.2	
410	723,000	18,000	40.2	50,000	2.8	
411	*750,000*	*240,000*	*3.1*	*156,000*	....	
412	*760,000*	*60,000*	*12.7*	*44,000*	....	
413	760,000	60,000	12.7	127,000	2.1	
414	774,000	33,000	23.5	155,000	4.7	
415	792,000	36,000	22.0	139,000	3.9	
416	*795,000*	*30,000*	*26.5*	*29,000*	....	
417	815,000	110,000	7.4	160,000	1.5	
418	*830,000*	*100,000*	*8.3*	*98,000*	....	
419	*850,000*	*320,000*	*2.7*	*59,000*	....	
420	860,000	30,000	28.7	38,000	1.3	
421	882,000	54,000	16.6	140,000	2.6	
422	890,000	120,000	7.4	855,000	7.1	
423	900,000	90,000	10.0	133,000	1.5	
424	906,000	84,000	10.5	590,000	7.0	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*)

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
425	*910,000*	*105,000*	*8.7*	*51,000*	....	
426	930,000	30,000	31.0	83,000	2.8	
427	942,000	51,000	18.5	162,000	3.4	
428	951,000	21,000	45.3	61,000	2.9	
429	1,000,000	105,000	9.5	453,000	4.3	
430	1,008,000	63,000	16.0	164,000	2.6	
431	1,030,000	200,000	5.2	513,000	2.6	
432	1,030,000	90,000	11.4	265,000	2.9	
433	*1,089,000*	*78,000*	*14.0*	*49,000*	....	
434	1,120,000	200,000	5.6	324,000	1.6	
435	*1,120,000*	*250,000*	*4.5*	*193,000*	....	
436	1,128,000	54,000	20.9	92,000	1.7	
437	1,135,000	70,000	16.2	133,000	1.9	
438	1,140,000	70,000	16.3	335,000	4.8	
439	1,140,000	114,000	10.0	144,000	1.3	
440	1,140,000	210,000	5.4	2,643,000	....	
441	1,180,000	90,000	13.1	151,000	1.7	
442	1,194,000	63,000	18.9	128,000	2.0	
443	*1,220,000*	*200,000*	*6.1*	*172,000*	....	
444	1,260,000	260,000	4.8	690,000	2.7	
445	1,272,000	72,000	17.7	203,000	2.8	
446	1,300,000	105,000	12.4	1,113,000	10.6	
447	1,310,000	65,000	2.0	151,000	2.3	
448	1,490,000	250,000	6.0	297,000	1.2	
449	1,560,000	140,000	11.2	351,000	2.5	
450	*1,590,000*	*450,000*	*3.5*	*3,350*	....	
451	1,600,000	370,000	4.3	513,000	1.4	
452	1,620,000	370,000	4.4	1,140,000	3.1	
453	1,680,000	170,000	9.9	267,000	1.6	
454	*1,920,000*	*330,000*	*5.8*	*294,000*	....	
455	1,940,000	250,000	7.8	523,000	2.1	
456	1,960,000	100,000	19.6	287,000	2.9	
457	2,020,000	180,000	11.2	483,000	2.7	
458	2,080,000	590,000	3.5	600,000	1.0	Crowded plates.
459	2,400,000	380,000	6.3	1,500,000	3.9	
460	2,550,000	630,000	4.0	1,580,000	2.5	
461	2,630,000	440,000	6.0	760,000	1.8	
462	2,650,000	170,000	15.6	1,000,000	5.9	
463	2,802,000	162,000	17.3	586,000	3.6	
464	2,830,000	170,000	16.6	530,000	3.1	
465	2,940,000	190,000	15.5	224,000	1.2	
466	2,960,000	520,000	5.7	580,000	1.1	
467	3,020,000	310,000	9.7	2,500,000	8.1	
468	3,120,000	20,000	156.0	59,000	3.0	
469	3,210,000	240,000	13.4	860,000	3.6	

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[1] All counts per cubic centimeter of milk.

TABLE I.— COLONIES[1] ON PLAIN AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*concluded*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
470	3,240,000	180,000	18.0	631,000	3.5	
471	3,770,000	240,000	15.7	1,016,000	4.2	
472	4,080,000	220,000	18.4	933,000	4.2	Crowded plates.
473	4,560,000	580,000	7.7	1,685,000	2.9	
474	*5,080,000*	*200,000*	*25.4*	*111,000*	....	
475	*5,330,000*	*1,410,000*	*3.8*	*903,000*	....	
476	5,380,000	1,010,000	5.3	3,053,000	3.0	
477	6,080,000	610,000	9.6	1,073,000	1.8	
478	*6,220,000*	*670,000*	*9.3*	*47,000*	....	
479	6,270,000	2,130,000	2.9	2,800,000	1.3	
480	*6,540,000*	*1,155,000*	*5.7*	*1,143,000*	....	
481	*6,930,000*	*5,310,000*	*1.3*	*58,000*	....	
482	*7,030,000*	*390,000*	*18.0*	*88,000*	....	
483	7,820,000	2,620,000	3.0	2,820,000	1.1	
484	15,240,000	3,220,000	4.8	3,515,000	1.1	
485	26,280,000	4,800,000	5.5	8,856,000	1.8	Crowded plates.
486	30,180,000	5,880,000	5.1	10,650,000	1.8	Crowded plates.
487	*33,840,000*	*11,440,000*	*3.0*	*8,426,000*	....	Crowded plates.
488	49,320,000	6,880,000	7.2	12,377,000	1.8	Crowded plates.
489	51,240,000	3,680,000	13.9	7,013,000	1.9	Crowded plates.
490	*68,820,000*	*21,660,000*	*3.2*	*4,380,000*	....	Crowded plates.
491	*73,440,000*	*21,720,000*	3.4	*7,145,000*	....	Crowded plates.
Averages....		....	8.1	....	2.6	

TABLE II.— COLONIES[1] ON LACTOSE AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD.

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
1	Less than 3,000 per c. c.		....	5,670	....	Less than 20 colonies per plate.
2	Less than 3,000 per c. c.		....	6,000	....	Less than 20 colonies per plate.
3	Less than 3,000 per c. c.		....	8,300	....	Less than 20 colonies per plate.

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[1]All counts per cubic centimeter of milk.

TABLE II.— COLONIES[1] ON LACTOSE AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
4	Less than 3,000 per c. c.		....	12,000	....	
5	Less than 3,000 per c. c.		....	14,000	....	
6	Less than 3,000 per c. c.		....	19,000	....	
7	Less than 3,000 per c. c.		....	21,000	....	
8	Less than 3,000 per c. c.		....	24,000	....	
9	Less than 3,000 per c. c.		....	29,000	....	
10	Less than 3,000 per c. c.		....	29,000	....	
11	Less than 3,000 per c. c.		....	39,000	....	
12	*6,000*	*6,000*	*1.0*	*4,630*	....	
13	*9,000*	*6,000*	*1.5*	*4,100*	....	
14	12,000	3,000	4.0	5,830	1.9	
15	12,000	6,000	2.0	7,000	1.2	Less than 20 colonies per plate.
16	12,000	6,000	2.0	7,830	1.3	
17	12,000	6,000	2.0	8,000	1.3	Less than 20 colonies per plate.
18	12,000	6,000	2.0	8,070	1.3	
19	12,000	6,000	2.0	17,000	....	
20	12,000	6,000	2.0	20,000	....	
21	12,000	6,000	2.0	25,000	....	
22	12,000	6,000	2.0	26,000	....	
23	12,000	6,000	2.0	32,000	....	
24	12,000	6,000	2.0	156,000	....	
25	*18,000*	*12,000*	*1.5*	*7,500*	....	
26	*18,000*	*12,000*	*1.5*	*12,000*	....	Less than 20 colonies per plate.
27	18,000	12,000	1.5	44,000	....	
28	21,000	3,000	7.0	12,000	4.0	
29	21,000	9,000	2.3	26,000	....	
30	24,000	3,000	8.0	34,000	....	
31	24,000	6,000	4.0	6,330	1.1	Less than 20 colonies per plate.
32	24,000	9,000	2.7	10,000	1.1	
33	*24,000*	*12,000*	*2.0*	*9,670*	....	
34	*30,000*	*24,000*	*1.3*	*11,000*	....	Less than 20 colonies per plate.
35	*36,000*	*12,000*	*3.0*	*9,000*	....	
36	36,000	18,000	2.0	32,000	1.8	
37	42,000	12,000	3.5	116,000	....	
38	*42,000*	*24,000*	*1.8*	*3,670*	....	
39	*42,000*	*24,000*	*1.8*	*9,660*	....	
40	45,000	21,000	2.1	33,000	1.6	
41	48,000	3,000	16.0	12,000	4.0	
42	48,000	6,000	8.0	20,000	3.3	
43	48,000	6,000	8.0	31,000	5.2	

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[1] All counts per cubic centimeter of milk.

TABLE II.— COLONIES[1] ON LACTOSE AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	Microscopic Method.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
44	48,000	24,000	2.0	25,000	1.0	
45	60,000	6,000	10.0	40,000	6.7	
46	60,000	12,000	5.0	13,000	1.1	
47	60,000	12,000	5.0	14,000	1.2	Less than 20 colonies per plate.
48	60,000	12,000	5.0	135,000	....	
49	*60,000*	*24,000*	*2.5*	*7,000*	....	Less than 20 colonies per plate.
50	66,000	18,000	3.4	49,000	2.7	
51	72,000	12,000	6.0	160,000	....	
52	*78,000*	*18,000*	*4.3*	*9,500*	....	
53	*84,000*	*18,000*	*4.7*	*7,400*	....	
54	84,000	36,000	2.3	65,000	1.8	
55	90,000	30,000	3.0	73,000	2.4	
56	*96,000*	*12,000*	*8.0*	*11,000*	....	Less than 20 colonies per plate.
57	96,000	24,000	4.0	39,000	1.6	
58	*96,000*	*48,000*	*2.0*	*40,000*	....	
59	108,000	18,000	6.0	31,000	1.7	
60	*120,000*	*18,000*	*6.7*	*13,000*	....	
61	*126,000*	*18,000*	*7.0*	*14,000*	....	Less than 20 colonies per plate.
62	126,000	42,000	3.0	194,000	....	
63	138,000	36,000	3.8	154,000	....	
64	141,000	27,000	5.2	65,000	2.4	
65	144,000	48,000	3.0	107,000	2.2	
66	156,000	18,000	8.7	69,000	3.8	
67	168,000	30,000	5.6	32,000	1.1	
68	168,000	42,000	4.0	124,000	3.0	
69	174,000	24,000	7.2	43,000	1.8	
70	195,000	45,000	4.3	240,000	....	
71	200,000	80,000	2.5	98,000	1.2	
72	210,000	48,000	4.4	53,000	1.1	
73	216,000	24,000	9.0	41,000	1.7	
74	216,000	36,000	6.0	112,000	3.1	
75	240,000	27,000	8.9	66,000	2.4	
76	246,000	15,000	16.4	25,000	1.7	
77	258,000	48,000	5.4	52,000	1.1	
78	264,000	6,000	44.0	19,000	3.2	Less than 20 colonies per plate.
79	276,000	36,000	7.7	74,000	2.1	
80	280,000	60,000	4.7	199,000	3.3	
81	288,000	36,000	8.0	111,000	3.1	
82	310,000	110,000	2.8	365,000	....	

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[1] All counts per cubic centimeter of milk.

TABLE II.— COLONIES[1] ON LACTOSE AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*continued*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
83	*312,000*	*12,000*	*26.0*	*5,670*	....	Less than 20 colonies per plate.
84	318,000	24,000	13.2	65,000	2.7	
85	330,000	90,000	3.7	161,000	1.8	
86	342,000	36,000	9.5	67,000	1.9	
87	348,000	42,000	8.3	147,000	3.5	
88	*384,000*	*18,000*	*21.3*	*6,000*	....	Less than 20 colonies per plate.
89	400,000	80,000	5.0	277,000	3.5	
90	402,000	36,000	11.2	79,000	2.2	
91	410,000	70,000	5.8	242,000	3.5	
92	450,000	60,000	7.5	171,000	2.9	
93	498,000	54,000	9.2	97,000	1.8	
94	510,000	160,000	3.2	178,000	1.1	
95	528,000	78,000	6.8	523,000	6.7	
96	540,000	80,000	6.8	186,000	2.3	
97	546,000	48,000	11.4	72,000	1.5	
98	558,000	78,000	7.2	111,000	1.4	
99	*588,000*	*60,000*	*9.8*	*41,000*	....	
100	600,000	80,000	7.5	295,000	3.6	
101	600,000	145,000	4.2	302,000	2.0	
102	654,000	114,000	5.7	323,000	2.8	
103	714,000	84,000	8.5	320,000	3.8	
104	732,000	66,000	11.1	313,000	4.7	
105	750,000	36,000	20.8	97,000	2.7	
106	768,000	66,000	11.6	178,000	2.7	
107	798,000	144,000	7.0	178,000	1.2	
108	798,000	60,000	13.3	187,000	3.1	
109	*800,000*	*200,000*	*4.0*	*196,000*	....	
110	*845,000*	*650,000*	*1.3*	*457,000*	....	
111	858,000	96,000	8.9	129,000	1.3	
112	882,000	90,000	9.8	226,000	2.5	
113	910,000	105,000	8.7	280,000	2.7	Crowded plates.
114	910,000	130,000	7.0	225,000	1.7	
115	1,032,000	42,000	24.6	110,000	2.6	
116	1,120,000	240,000	4.6	687,000	2.9	
117	*1,200,000*	*160,000*	*7.5*	*124,000*	....	
118	1,200,000	240,000	5.0	400,000	1.7	
119	1,260,000	220,000	5.7	413,000	1.9	
120	1,360,000	110,000	12.4	222,000	2.0	
121	*1,360,000*	*240,000*	*5.7*	*203,000*	....	
122	1,380,000	180,000	7.7	887,000	4.9	
123	1,482,000	60,000	24.7	192,000	3.2	
124	1,494,000	90,000	16.6	168,000	1.9	

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[1] All counts per cubic centimeter of milk.

TABLE II.— COLONIES[1] ON LACTOSE AGAR, AND BACTERIA[1] FOUND BY THE DIRECT MICROSCOPIC METHOD (*concluded*).

Sample No.	MICROSCOPIC METHOD.			Number colonies on agar plates.	Average breaking up of "groups."	Notes.
	Number of individual bacteria.	Number of "groups."	Average size of "groups."			
125	1,506,000	6,000	251.0	17,000	2.8	Less than 20 colonies per plate.
126	1,548,000	108,000	14.3	333,000	3.1	
127	1,560,000	220,000	7.1	360,000	1.6	
128	1,660,000	360,000	4.6	1,163,000	3.2	
129	*1,720,000*	*380,000*	*4.5*	*164,000*	....	
130	*1,790,000*	*220,000*	*8.1*	*194,000*	....	
131	*1,850,000*	*280,000*	*6.6*	*246,000*	....	
132	2,010,000	170,000	11.2	248,000	1.4	
133	2,100,000	720,000	2.9	1,020,000	1.4	
134	2,700,000	540,000	5.0	1,146,000	2.1	
135	2,740,000	660,000	4.2	903,000	1.4	
136	3,500,000	400,000	8.8	500,000	1.3	
137	3,540,000	520,000	6.8	897,000	1.7	
138	3,880,000	620,000	6.3	1,030,000	1.7	
139	4,150,000	600,000	6.9	745,000	1.2	
140	4,320,000	960,000	4.5	1,140,000	1.2	
141	5,190,000	570,000	9.1	603,000	1.1	
142	5,560,000	390,000	14.3	1,300,000	3.3	
143	5,940,000	570,000	10.4	1,310,000	2.3	
144	*7,280,000*	*390,000*	*18.7*	*119,000*	....	
145	7,680,000	260,000	29.5	510,000	2.0	
146	*8,040,000*	*1,740,000*	*4.6*	*1,703,000*	....	
147	8,400,000	1,050,000	8.0	1,817,000	1.7	
148	8,600,000	300,000	28.7	2,670,000	8.9	
149	8,940,000	960,000	9.3	8,900,000	9.3	
150	10,610,000	970,000	10.9	7,500,000	7.7	
151	14,070,000	3,420,000	4.1	3,833,000	1.1	
152	19,800,000	6,420,000	3.1	9,410,000	1.5	Crowded plates.
Averages....		....	8.9		2.6	

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[1] All counts per cubic centimeter of milk.

CLASS I. ONE HUNDRED AND SEVENTY-FIVE SAMPLES (27 PER CT.) WHICH GAVE PLATE COUNTS GREATER THAN THE INDIVIDUAL COUNT BY THE MICROSCOPE.

In this type of counts it is self-evident that the individual counts must be smaller than they should be or else the plate counts are larger than they should be. The individual counts might be smaller for either of two reasons. First, the bacteria where they are very few in number, even tho well stained and conspicuous may not occur in microscope fields examined; a condition which probably occurs, as is suggested by the fact that so many counts of this class are grouped among those samples containing few bacteria. Second, the bacteria may be overlooked thru carelessness or because they are exceptionally tiny or because they do not stain well. The plate counts, on the other hand, would be too large in any case where contaminations took place during the process of plating.

Errors due to unequal distribution of the bacteria in the original milk samples, difficulties involved in measurements and the like may explain some of the discrepancies which are not too great. The probability is, however, that bacteria have been overlooked in those smears containing few bacteria as only a small portion of the smear was examined. The possibility that this has happened becomes quite apparent if one considers the mathematical chances of not finding the bacteria in the smear. For instance, suppose a smear had only one organism on it, that is, 100 bacteria per cubic centimeter, and the microscope was so adjusted that there were 3000 microscopic fields on the smear. If 100 fields were examined (and rarely were more than this examined) then only $\frac{1}{30}$ of the whole smear would be studied. This makes the chance of finding the single organism one in thirty. In other words, in 29 cases the microscopic count would be too low as compared to the plate count, while in the one case it would be too high.

In view of these mathematical relationships it is not surprising to find that the majority of the counts of this type were found in milks containing few bacteria.

Only a very few instances have been noted in which errors have been caused by the presence of tiny or lightly stained bacteria. It is well known that methylene blue normally stains practically all bacteria deeply; and very rarely, in any systematic study of milk flora, have any species been described which are too small to see plainly with the magnification used in this work. However the fact that very tiny bacteria were isolated from samples 24, 48 and 51 (Table II), all of which came from the same dairy, show that small size is a factor which occasionally causes trouble.

It is known that contamination frequently takes place during the plating of samples of milk. The extraneous colonies may come from the dust in the air, in which case the number of extra colonies is not large per plate; but they may, nevertheless, be sufficient in number to cause the plate count to be higher than the individual count; or, the extraneous colonies may come from unsterile media, dilution waters, glassware and the like in which case the number of colonies added may be very large and therefore make the error great. In spite of the care used in preparing the plates for this work it is scarcely probable that all contaminations were avoided. Undoubtedly this is the reason why some of the plate counts in class I are larger than the individual counts.

There are two instances in particular (samples Nos. 131 and 367, Table I) where the discrepancy is so great that it can scarcely be explained in any other way. In the first of these cases, the total number of individual bacteria counted under the microscope was only 15,000 per c. c. In spite of this, all of the plates checked up well and the three which were counted showed an average of 149.3 colonies per plate, the count being 1,493,000 per c. c. Likewise in sample 357, the total number of bacteria found under the microscope showed only 282,000 per c. c., while the plates which were perfectly normal in every respect gave a count of 1,477,000 per c. c. A careful recounting of the microscopic smears and an examination under a higher power lens failed to change the original microscopic counts to any material extent.

CLASS II. ONE HUNDRED AND TWENTY-THREE SAMPLES (19 PER CT.) WHICH GAVE PLATE COUNTS LESS THAN THE "GROUP" COUNT BY THE MICROSCOPE.

It is evident that the plate counts in Class II are smaller than they should have been, tho in many of these cases the discrepancy is so small that it was probably caused by ordinary experimental errors. There are, however, two possible sources of discrepancies of this type which should be considered. They are, first, the presence of living bacteria in milk which do not grow on agar, and second, the presence of dead bacteria.

It is a well known fact that, among the bacterial species commonly found in milk, there exist certain types which are alive and vigorous but which are not adapted to growth in the cultural media ordinarily used in routine milk work. These include some pathogens, such as the tubercle organism, concerning which Ostertag[9] claims to have transmitted the disease to animals by injecting a $\frac{1}{1,000,000}$ dilution of milk, and the *Bact. abortus*-like bacillus recently described by Evans.[10] She reports as many as 112,000 of these organisms per cubic centimeter in milk at the time of drawing from the udder and also that this organism does not grow on ordinary agars. Among the milk organisms which commonly do not grow or grow poorly on agar are also the long-rod, lactic acid group, strict anaerobes, and possibly some others. The presence of bacteria belonging to these types might cause the plate count to be smaller than the "group" count.

It is clear that if milk contains a large number of dead bacteria in stainable condition the plate count will be lower than the "group" count. However, only 19 per ct. of the 643 samples analyzed gave plate counts lower than the "group" count. Since many of these were due to experimental errors and also in view of the known presence of live bacteria which do not grow in agar, it is evident that the presence of large numbers of dead bacteria in unpasteurized milk, is the exception rather than the rule.

In class II as in class I there are also some discrepant counts which deserve special consideration, the most striking of which have been copied in Table III.

TABLE III.—COMPARATIVE COUNTS FROM FIVE SAMPLES OF MILK IN WHICH PLATE COUNTS WERE LOWER THAN "GROUP" COUNTS.

Selected from Table I.

Sample No.	MICROSCOPIC METHOD.		No. of colonies on agar plates.
	No. of individual bacteria.	No. of "groups" of bacteria.	
419	850,000	320,000	59,000
450	1,590,000	450,000	3,350
478	6,220,000	670,000	47,000
481	6,930,000	5,310,000	58,000
482	7,030,000	390,000	88,000

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[9] Ostertag, Robert von. Die Bekämpfung der Tuberculose des Rindes. XXI+591 pp., 88 figs., Berlin. 1913.

[10] Evans, Alice C. The large numbers of Bact. abortus, var. lipolyticus which may be found in milk. *Jour. Bact.*, **2**:185–186. 1917.

Recounts of the smears showed them actually to contain approximately the number of bacteria found in making the first microscopic counts. The bacteria in the smears were apparently of the same nature as those found in other smears where the counts agreed better.

If the discrepancies were caused by failure of living bacteria to grow, the plate counts give a very misleading idea of the bacterial quality of the milk. If on the other hand dead bacteria were present, caused possibly by the refrigeration of the milk or by the addition of preservatives to the milk, neither count alone gives an entirely adequate idea of the conditions tho the microscopic count would come nearer to doing so than would the plate count.

CLASS III. THREE HUNDRED AND FORTY-FIVE COUNTS IN WHICH THE PLATE COUNT WAS GREATER THAN THE "GROUP" COUNT AND LESS THAN THE INDIVIDUAL COUNT BY THE MICROSCOPE.

According to the results obtained in this work comparative counts of the colonies of bacteria in milk made by the plate method and the two counts as made by the direct microscopic method can be expected to show this normal theoretical relationship in 54 per ct. of the cases. This percentage is increased to 71 when the number of bacteria in the milk is 30,000 per cubic centimeter or more. A study of the counts in Tables I and II reveals the fact that there is considerable variation in the relations between the "group" and individual counts and the plate count. The plate count may be very nearly the same as the "group" count or it may be very nearly the same as the individual count.

There is little question but that the same errors which caused the discrepancies in the counts discussed under classes I and II exert some influence in the case of those samples in which the three counts show this normal theoretical relationship. Some samples may contain dead organisms, others may contain tiny or light-staining organisms while others may contain living bacteria which cannot grow on agar; or bacteria of all three kinds may exist in the same sample, in which case one error tends to counterbalance the others. Outside of these things the "group" with its variations in size and compactness is the principal factor which determines the ratio between the plate count and the two counts obtained by the microscopic method.

A study was therefore made of the number of bacteria in the "groups" found normally in market milk. This has been done in two ways; First, a study was made of the size and character of the organisms in "groups" selected at random from 1440 samples of market milk. Second, the size of the "groups" found in the 643 samples of milk which were used in making the comparative counts was noted.

The size and character of the "groups" in smears made from market milk.—Since it had been generally observed that as the number of bacteria in market milks increased, the average size of the "groups" decreased, a separate study of the bacteria in milks of different grades was made. The 1440 smears examined were selected at random from among 15,000 smears made from a like number of samples of market milk. These preparations had been previously examined and classified into grades designated as A, B and C. The bacterial limits for each grade were based upon the individual count by the microscope and were as follows: Grade A, all samples containing less than 1,000,000 individual bacteria per cubic centimeter; Grade B, all samples containing between 1,000,000 and 10,000,000 bacteria per cubic centimeter; Grade C, all samples containing more than 10,000,000 bacteria per cubic centimeter.

Twelve hundred "groups" of bacteria were studied in each grade. The bacteria seen were classified as follows: Streptococci, *Bacterium lactis acidi?* micrococci and rods, all of which are commonly found in milk. The procedure in the case of the Grade A milks, where the bacteria were few in number, was to make a record of the number of individuals in the first group found and to classify as above. Only one source was studied from each smear and the smear was passed by if none could be found in fifty fields. Accordingly the 1200 "groups" found in Grade A milks represent studies made on 1200 samples of milk containing enough bacteria so that at least one "group" was found in the first fifty fields examined. The procedure in the case of both B and C milks, where the bacteria were more easily found was to record the number of individuals in each of the first ten "groups" seen and to classify as above. Therefore the 1200 "groups" recorded under B and C represent studies made on 120 samples of milk for each grade. In Grade C, where many bacteria were present in each field, the tendency to see the larger groups first was overcome by the use of an ocular micrometer which divided the field into small sectors and the same sector was used thruout the work. The results of these studies are summarized in Table IV.

TABLE IV.—AVERAGE SIZE OF "GROUPS" OF BACTERIA AS THEY EXIST IN MARKET MILK OF DIFFERENT GRADES.

Grade of milk.	Type of organism.	No. of "groups" seen.	Total No. bacteria seen.	Ave. size of "group"
A	Streptococci	110	5,354	48.6
B	Streptococci	221	5,611	25.4
C	Streptococci	121	3,358	27.8
All grades	Streptococci	452	14,323	33.7
A	Bact. lactis acidi?*	22	76	3.5
B	Bact. lactis acidi?*	47	184	3.2
C	Bact. lactis acidi?*	142	357	2.5
All grades	Bact. lactis acidi?*	211	617	3.1
A	Micrococci	539	9,478	17.6
B	Micrococci	481	6,214	12.9
C	Micrococci	352	4,217	12.0
All grades	Micrococci	1,372	19,909	14.2
A	Rods	529	2,947	5.6
B	Rods	450	2,812	6.3
C	Rods	585	2,148	3.7
All grades	Rods	1,564	7,907	5.2
B	Yeasts	1	6	6.0
A	All types	1,200	17,855	14.9
B	All types	1,200	14,827	12.2
C	All types	1,200	10,080	8.4
All grades	All types	3,600	42,762	11.8

*As determined by microscopic examination only.

The two things which stand out most prominently in Table IV are the great differences in the average size of the "groups" of different types of bacteria, and the frequent decrease in the average size of the "groups" in samples containing large numbers of bacteria as compared to samples containing fewer bacteria. The latter condition shows most plainly in the average size of "groups" found for all types of organisms.

It will be noticed that the "groups" of streptococci are consistently larger than those of any of the other types. At the same time, according to the results here found, they appear to grow in somewhat smaller " groups " in samples containing large numbers of bacteria than in milk containing fewer bacteria. However, the results of studies of pure cultures of streptococci in milk, summarized in Table VII, Part II (page 237), indicate that this decrease in size of " groups " as the number of bacteria increases is not invariable. The average size of the " groups " of streptococci from the market milks was 33.7, while in the strain studied in pure culture these " groups " included an average of 55.0 individuals.

The micrococci showed " groups " which averaged next in size to those of the streptococci. The average " group " of micrococci in all grades of milk was found to include 14.2 individuals. In this case, again, there was a tendency for the "groups" to decrease in size in the samples containing large numbers of bacteria.

The rod types grow in much smaller " groups," the average size of " groups " being 5.2. The size of the " groups " varied from an average of 6.3 in the Grade B samples to 3.7 in the Grade C samples.

As would be expected, the average size of the " groups " of bacteria classified as *Bacterium lactis acidi* remains about the same in all three grades of milk, varying from 2.5 to 3.5, with an average number of 3.1 individuals per " group." The most interesting thing to note in connection with these organisms is that they become relatively more abundant in the milks containing large numbers of bacteria. Only 22 " groups " including organisms of this type were noted among the 1200 " groups " studied from Grade A milk, 47 " groups " were found in the Grade B milks and 142 in the Grade C milks. As the organisms of the *Bacterium lactis acidi* type increase in numbers there is a decrease in the number of " groups " of micrococci.

A single " group " including six individual yeast cells was noted in one instance. Yeasts occur but rarely in market milks.

In the lower part of the last column of Table IV, the average size of " group " is given for all of the bacteria seen in each of the three grades of milk. It will be seen from the averages that there is a gradual decrease in the size of the " groups " as the grade of the milk passes from A to C. That is, in the Grade A milk, the average size of " group " is 14.9. This becomes 12.2 in the Grade B milk, and 8.4 in the Grade C milk. In considering the figures it should be kept in mind that in selecting the " groups " from the Grade A milk it was necessary to disregard all samples in which no bacteria were found in the first 50 fields of the microscope (" group " count less than 6000 per c. c.). Since all of this milk was fresh market milk (less than 16 hours old in all cases), even the Grade C milk includes very little milk that was approaching the souring point, or milk in which the bacteria had had a chance to grow for any length of time.

The average size of the " groups " found in 580 of the samples of milk used for the comparative counts given in Tables I and II.—The fact that it is possible to compute the average size of the " groups " of bacteria present in any given sample of milk by dividing the individual count by the " group " count, makes it possible to study the size of the " groups " found in 580 of the 643 samples which were used for the comparative counts. The results obtained from these computations have been averaged and summarized in Table V.

TABLE V.—AVERAGE SIZE OF THE " GROUPS " OF BACTERIA FOUND IN 580 OF THE SAMPLES OF MARKET MILK USED IN SECURING THE RESULTS IN TABLES I AND II.

Grade of milk.	No. bacteria as determined by microscopic examination.	No. samples examined	Average size of " group "
A....	Under 30,000 per c. c	137	2.2
	30,000 to 200,000	203	6.7
	200,000 to 1,000,000	139	12.9
B....	1,000,000 to 10,000,000	89	*11.9
C....	Over 10,000,000 per c. c	11	5.6
	Total	579	Ave. 7.9

* Does not include one abnormal count (No. 125, Table II) where the average size of group was 251.

As previously stated, the samples classified as Grade A included all milks having less than 1,000,000 bacteria per cubic centimeter. However in order to study the size of the "groups" in samples containing few bacteria, this grade was further divided into three classes as follows: (1) Those samples having less than 30,000 individual bacteria per cubic centimeter, (2) those having between 30,000 and

200,000 per cubic centimeter and (3) those having between 200,000 and 1,000,000 per cubic centimeter.

This subdivision of the Grade A milk into three classes brings out a surprising and interesting fact, as will be seen from the figures given in the last column of Table V. Here it appears that the average "group" of bacteria in the 137 samples of milk which contained less than 30,000 bacteria per c. c. included but 2.2 individuals. In the 203 samples of milk which contained between 30,000 and 200,000 bacteria per c. c., there is an increase in the size of the "groups" to 6.7. The "groups" increase still more in size in the 139 samples of milk which contained between 200,000 and 1,000,000 bacteria per c. c. The average size noted in this case (12.9) compares well with that noted for the Grade A milk, Table IV, where the average size of the "groups" was 14.9.

From this point on the figures given in Table V indicate a decrease in size of the "groups" that corresponds closely to that recorded in Table IV for the similar grades of milk. Thus for the Grade B milk in Table IV, the size of the "groups" is given as 12.2 while in Table V the similar milk shows an average size of the "groups" of 11.9. Similarly the Grade C milk in Table IV shows an average size of the "groups" of 8.4, while in Table V, the figures given for similar milk are 5.6.

The samples used in obtaining these averages represent but one type of market milk so that the data given are not extensive enough to justify the claim that the conditions noted are universal. It is therefore not worth while to speculate on the reasons for these increases and decreases in the average size of the "groups." Enough has been seen, however, to justify the statement that the average size of the "groups" is very largely dependent upon the predominant type of organism present, and probably also somewhat dependent upon the conditions under which the organism is growing.

The cooperative investigations reported by Breed and Stocking[11] furnish counts made upon milks containing known types of organisms which give some idea of the size of groups to be expected with various types of bacteria. Some of these samples were inoculated with pure cultures of different organisms, while some were selected from market milk known to contain a predominant flora of long-chain streptococci. The results summarized in Table VI are selected from their publication.

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[11] See footnote 4.

TABLE VI.— COUNTS SELECTED FROM BREED AND STOCKING REPORT WHICH ILLUSTRATE THE INFLUENCE OF THE TYPE OF ORGANISM ON THE SIZE OF THE "GROUPS."

No.	MICROSCOPIC COUNT.		No. colonies on agar plates	Average size of "group."	Notes.
	Number of individual bacteria.	Number of "groups" of bacteria.			
1	2,680,000,000	1,531,000,000	1,982,000,000	1.7	Milk nearly ready to curdle. Pure culture Bact. lactis acidi.
2	99,480,000	93,480,000	51,550,000	1.1	Milk inoculated with long rod lactic acid bacteria from cheese whey.
3	67,450,000	49,900,000	47,600,000	1.3	Milk inoculated with colon bacillus.
4	1,570,000	995,000	1,381,000	1.6	Milk inoculated with colon bacillus.
5	809,000	513,000	609,000	1.6	Milk inoculated with colon bacillus.
6	476,000	262,000	397,000	1.8	Milk inoculated with colon bacillus.
7	150,200,000	5,930,000	9,867,000	25.3	Milk containing masses of long chain streptococci.
8	233,000	12,600	25,700	18.6	Milk containing masses of long chain streptococci.

From Table VI it will be seen that some organisms such as the lactic acid, or colon types grow normally in small groups averaging less than two individuals, while the streptococci grow normally in larger groups which may vary considerably in size.

Influence of the size of the "groups" upon the bacterial counts.— As has been stated on page 229, the fact that bacteria in milk occur in clumps which may or may not be readily broken apart into their component individuals is the chief factor which affects the plate counts included in class III.

In making counts by the microscopic method, the occurrence of the bacteria in clumps of unequal sizes rather than as isolated individuals makes the distribution of the individual bacteria in the microscopic preparations somewhat irregular. Therefore in order to secure a reliable count it is necessary to examine a larger quantity of milk than would otherwise be necessary. This unevenness of distribution may be so great that the labor involved in making an examination of a sufficiently large quantity of milk to make the count a reliable one is prohibitive. In such cases the microscopic count becomes an " estimate " rather than a " count " and may be quite inaccurate.

Occasionally such large and compact clumps of bacteria are present that it is quite impossible to determine the exact number of individuals in the clump even by the most careful microscopic examination. In such cases it is necessary to resort to estimates of the number present. Likewise, the border line between a single dividing individual and a pair of individuals is never a sharp one. Both of these conditions tend to make the microscopic count of individuals less accurate than it otherwise would be.

Under such conditions it is to be expected that the most accurate microscopic counts can be made from samples where the bacteria exist entirely or largely as isolated individuals.

The size of the " groups " has a very pronounced influence upon the significance and interpretation of the meaning of the colony count by the plate method. This is shown in Table VII which contains a summary of comparative counts made from samples inoculated with pure cultures of two different organisms which characteristically grow in different sized " groups."

The table includes two series of counts, one made from a sample of milk inoculated with a culture of *Bact. aerogenes*, and the other with a culture of a long chain streptococcus derived from the udder. In the sample inoculated with *Bact. aerogenes*, individual, " group " and plate counts were made at hourly intervals for 13 hours, after which the sample stood over night (8 hours) and was again examined twice with an hour interval between the two examinations. The second sample was examined at hourly intervals for 7 hours. The samples were incubated at a temperature of 21° C.

As will be seen from the results given in Part I, Table VII, the plate counts made from the milk containing *Bact. aerogenes* (an organism which grows well on agar, and which exists in milk cultures largely as isolated individuals or as pairs) are ordinarily a little less

Table VII.—Part I. Microscopic and Plate Counts Made from a Sample of Milk Inoculated with *Bact. aerogenes.*

No.	Microscopic Count.		Average size of "group."	No. colonies on agar plates.
	No. of individual bacteria per c. c.	No. of "groups" of bacteria.		
*1	51,000	35,000	1.5	41,000
2	53,000	32,000	1.7	40,000
3	87,000	51,000	1.5	38,000
4	39,000	39,000	1.0	20,000
5	39,000	30,000	1.3	29,000
6	126,000	84,000	1.5	20,000
7	129,000	63,000	2.0	23,000
8	46,000	39,000	1.2	22,000
9	117,000	30,000	3.9	13,000
10	197,000	30,000	6.5	24,000
11	171,000	57,000	3.0	57.000
12	129,000	42,000	3.1	93,000
13	4,260,000	1,215,000	3.5	1,570,000
14	9,105,000	2,100,000	4.3	4,115,000
*15	1,026,000,000	613,000,000	1.6	462,000,000
16	1,695,000,000	848,000,000	1.9	818,000,000

* Interval between analyses 14 and 15 was 8 hours. All others made at hourly intervals.

Table VII.—Part II. Microscopic and Plate Counts Made from a Sample of Milk Inoculated with a Long Chain Streptococcus from the Udder.

No.	Microscopic Count.		Average size of "group."	Number colonies on agar plates
	Number of individual bacteria per c. c.	Number of "groups." of bacteria.		
*1	801,000	24,000	33.3	48,000
2	896,000	20,000	44.8	154,000
3	2,109,000	63,000	33.4	115,000
4	5,328,000	87,000	61.2	290,000
5	6,885,000	117,000	58.8	541,000
6	10,262,000	147,000	69.8	665,000
7	16,520,000	245,000	67.4	685,000
8	60,270,000	840,000	71.3	843,000

* Analyses made at hourly intervals.

than the counts of the number of individual bacteria as seen under the microscope. On the other hand, the plate counts made from the milk containing the long-chain streptococcus (Part II) fail to give any adequate idea of the actual number of organisms present as they are invariably much smaller than are the counts of the individual bacteria as seen under the microscope. This happens because of the fact that the streptococci grow in large clumps which did not break up in the dilution waters. The plate counts obtained from the two samples at the beginning of the experiment were approximately the same (41,000 and 48,000 per c. c.), yet according to the counts made by the microscopic method one sample actually had about 33 times the number of bacteria found in the other sample. When the sample containing the streptococcus gave a plate count of 843,000 per c. c., the milk actually contained more bacteria than did the sample of milk containing *Bact. aerogenes* which gave a plate count of 4,115,000 per c. c. These counts bring out in a striking way the inadequacy of the plate as a method for counting individual bacteria.

If the bacterial flora of a sample of milk is made up of organisms which are found in "groups" containing from 1 to 4 individuals, all of which grow well on agar, the plate count may be expected to agree fairly well with, and be intermediate between, the microscopic "group" and individual counts. On the other hand if the flora is made up of organisms which grow well on agar but which grow in large compact groups, the difference between the plate count and the actual number of bacteria present is large. If the "groups" are so compact that they have not broken up during the plating process then the plate count will agree with the "group" count fairly well. If on the other hand the "groups" are not compact and break apart in the dilution waters, then the plate count will be more nearly the same as the individual count. Where plate counts alone are available it is never possible to determine which of the above conditions holds in the case of any particular sample so that it is quite impossible to tell whether the error in count is large or small.

Breaking apart of clumps.—If we assume that the "group" counts are reasonably accurate counts of the number of "groups" of bacteria originally present in a sample of milk, and that the plate counts are reasonably accurate counts of the "groups" after they have been broken apart in the dilution waters, then the ratio between the two counts will show the amount of breaking apart which the clumps have undergone in any given case. These ratios have therefore been computed for each of the 345 samples reported upon in Tables I and II which fall in Class III (bold faced type). Those counts which fall in Class I and II have been omitted in computing these ratios, for as explained on pages 225–9 there is reason for thinking that these counts are affected by sources of error having no relation to the size or the compactness of the clumps. The ratios have been given in column 6 of Tables I and II (pages 209–221). The average breaking apart of the "groups" was found to be 2.6 times, which would mean that the plate count in the average case is more than doubled by the breaking of the clumps. Individual cases were found where the " groups " broke apart a much greater number of times. In 24 instances the " groups " originally present broke into 5 or more smaller " groups " and in 12 instances into 7 or more smaller " groups." The greatest number of times the " groups " broke apart in any one sample was 16.5.

These figures indicate that two given samples may have the same number of individual bacteria, yet because there is a difference in the compactness of the clumps, the plate count on one might be 7 or even 16 times greater than on the other. Because of these variations, one sample might be rated as distinctly inferior in quality as compared to the other. Variations in compactness will be recognized as an inherent factor over which there is no control.

It has been generally observed that when the predominant bacteria in a given sample of milk grew in clumps in which the individuals appeared to be loosely arranged, the plate count would then be more nearly like the individual count by the microscope than like the " group " count. While on the other hand, if the predominating bacteria grew in closely compacted clumps, the plate count would be more nearly like the " group " count than the individual count.

The fact that it is the nature of the organisms present and the size and compactness of the clumps which has a determining influence upon the plate count (and not variations in methods of plating) is also indicated by the coöperative work reported by Breed and Stocking.[12] In this case six laboratory workers in two different laboratories secured practically identical counts in analyzing triplicate samples of milk inoculated with an organism of the colon group. Their conclusions are as follows:

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[12] See footnote 4.

"Where a milk contains nothing but living bacteria occurring singly (or at least with only a relatively small number of groups containing two or more individuals) all of which are capable of growth on the agar used, very consistent counts can be made by either method from duplicate samples by the same or by different persons. In such case the relation between the counts is such as to leave little doubt but that the figures obtained are remarkably accurate counts of the number of groups of bacteria in the case of both the microscopic and plate method; or of the number of bacteria present in the case of the microscopic method."

The question is often asked whether or not it is possible to predict what the plate count will be by means of a microscopic examination. It appears from the present studies that more than half of all of the samples gave plate counts intermediate between the "group" and individual counts. Over 70 per ct. of the samples containing more than 30,000 bacteria per c. c. showed this same relationship. These findings indicate that in about one-half of all cases, or in about 70 per ct. of milks containing more than 30,000 per c. c., one may reasonably expect the plate count to lie between the "group" and individual count. In the remainder of the cases where the count is affected the presence of bacteria which do not grow or by the presence of dead bacteria or where the count is inaccurate because the bacteria are too few, or too irregularly distributed to permit accurate microscopic counting, no constant relationship has been found to exist. Predictions as to plate counts are therefore frequently disappointing.

CONCLUSIONS.

In this investigation it has been found that the comparative counts made by the plate and microscopic methods on 643 samples of unpasteurized market milk fall into three classes. The first contains 175 samples (27 per ct.) in which there must have been some error in the count, as the plate count was greater than the individual count. All but 32 of these were found in samples which contained less than 30,000 bacteria per c. c. The grouping of so many of the counts of this type in low count milk indicates that the chief source of error in this case is in the microscopic count, and is due to the fact that such a small quantity of milk was examined in proportion to the number of bacteria present that the few present did not appear in the microscopic fields examined. On the other hand the distribution of the 32 counts of this type among the higher count milk indicates that some of the errors arose from unrecognized contaminations on the plates.

The second class contains 123 samples (17 per ct.) in which the plate counts were less than the " group " counts. In this class, it is also evident that there must have been errors of considerable size. As these occurred evenly distributed throughout the series of counts, it is evident that the cause of them must be something which affects all classes of milk. The most evident explanation and apparently the most probable is that in many of these cases bacteria were present which did not grow or grew poorly on ordinary agar. In some cases, the plate counts were probably low because of the presence of dead bacteria. The plate count in all of these cases gave a mistaken idea of the quality of the milk.

The third class contains 345 samples (54 per ct.) in which the plate counts are greater than the " group " count and less than the individual count. If however the 200 counts which were made from samples containing less than 30,000 bacteria per cubic centimeter are excluded, the number of instances in which the plate count is intermediate between the microscopic counts form 71 per ct. of the 443 counts which remain. Since comparative counts would naturally be expected to show this relationship, there is reason for thinking that these counts are not subject to the gross errors which were noted in the case of classes I and II. The plate count probably represents fairly well the number of " groups " of bacteria in the milk after they are broken apart by the dilution waters; the " group " count represents the number of " groups " of bacteria originally present in the milk; and the individual count represents the number of bacteria actually present. Under these conditions the variations in the size and compactness of the " groups " of bacteria originally present in the milk have the greatest influence in determining whether the plate count will be about the same as the " group " count or whether it will be more nearly equal to the individual count.

Market milk containing very few bacteria, the majority of which were presumably derived from udders which were not infected with streptococci, were found to show " groups " of bacteria of very small average size (2.2 individuals per " group "). There appears to be a marked increase in the average size of the " groups " in market milks containing larger numbers of bacteria, some of them long chain streptococci, others masses of bacteria washed off of the surfaces of utensils and like sources. The largest average size of the " groups " was found in market milks containing less than 1,000,000 bacteria per c. c. In milks containing larger numbers of bacteria there was a gradual decrease in the average size of the " groups " as the number of bacteria increased. The apparent reason for this decrease in the average size of the "groups" in high count milks is that there is a gradual change in the types of bacteria present. The longer the milk stands and the more growth there is, the more the bacteria originally present are overgrown by lactic acid types which grow normally in small " groups."

Variations in the size of the " groups " of bacteria affect the accuracy of the microscopic count because of the difficulty in counting the individuals in large, dense clumps and also because a few large clumps increase the error due to unevenness of distribution. The accuracy of the plate count is affected both because of the variations in the size of the " groups " and because the clumps do not entirely separate into their component individuals during the plating process.

The " groups " of bacteria in milk break apart on the average into 2.6 smaller " groups " in the process of preparing dilution waters. That is, under usual conditions the plate count will be increased from 2 to 3 times as a result of this factor alone. In individual cases, however, it may be increased more than this, the highest increase noted being 16.5 times.

The accuracy of the plate count is affected both because of the variation in the size of the " groups " and also because the clumps do not entirely separate into their component individuals during the plating process. The actual size of any plate count is largely the result of the interaction of these two highly variable conditions. Because no way exists in which the errors thus introduced can be discounted except by microscopic examination of the milk, the use of plate counts may result in great injustice being done where an attempt is made to make fine distinctions in the bacterial quality of milk.

Inasmuch as there are as yet no other more satisfactory methods in use for distinguishing between the bacterial quality of different samples of milk, it is fortunate that either of these methods (and this is more especially true of the microscopic method) can be used in a simpler way than is generally being done at the present time. The fact that accurate counts cannot be made does not prevent the use of either method as a means of distinguishing with a satisfactory degree of accuracy between milks containing few bacteria, a medium number, and a large number. The fact that this is all that is necessary in commercial or in control work has been shown in the inspection work which has been done for the City of Geneva during the past three years, a description of which will be given in a forthcoming bulletin.[13]

It is evident from all of this work that the current and popular ideas of the number of bacteria in milk based as they are upon counts made by the plating method must be revised. The results obtained by the microscopic method of counting bacteria in milk show that plate counts are not counts of the number of bacteria in milk as usually stated; but are counts of such groups of bacteria as grow upon agar at the incubation temperature used. Figures commonly given as showing the number of bacteria in milk are therefore ordinarily very much less than the actual number of bacteria present.

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[13] Breed, Robert S., and Brew, James D. N. Y. Agr. Exp. Sta., Bull. 443. 1917.

THE CONTROL OF BACTERIA IN MARKET MILK BY DIRECT MICROSCOPIC EXAMINATION.*

ROBERT S. BREED AND JAMES D. BREW.

SUMMARY.

1. A preliminary series of tests was made in a commercial bacteriological laboratory at Hobart, N. Y., in which the microscopic method of grading milk according to its bacterial quality was compared with the more generally used agar-plate method. The results secured from the agar-plate method at this laboratory were used as a basis of payment to dairymen, a premium being given for milk containing less than a specified number of bacteria.

2. The results of the comparative analyses showed that out of 1504 samples graded during the months of February, March and April, 1914, 1280 were graded A by the plate method and 1270 were graded A by the microscopic method. In 1339 instances there was an exact agreement in the grades given. In the case of 426 samples taken during July, 234 were graded A by the plate method and 212 were graded A by the microscopic method with an exact agreement in grade in 362 instances.

3. It was found to be impossible to get an exact agreement in results by the two methods as the plate count is not a count of the individual bacteria, but rather a count of the number of groups of bacteria as they exist after the groups are more or less broken apart by the shaking of samples and dilutions in the process of plating. The microscopic count, on the other hand, is either a count of the groups of bacteria as they originally existed in the milk, or is a count of the individual bacteria.

4. By a cooperative agreement with two milk companies in Geneva, the microscopic method was tried out during a period of twenty-six months for the purpose of grading the milk delivered into three grades. The results were used as a basis for payment to the dairymen.

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* Reprint of Bulletin No. 443, December, 1917.

5. No unforeseen difficulties were found in using the microscopic technique in this way. In all, 11,851 cans of milk were examined and classified in three grades. The entire work was done by one man who found it quite possible to handle from fifty to seventy samples each working day. Special precautions were taken to get accurate results by both the plate and the microscopic methods in a series of 643 comparative analyses. The results showed an exact agreement in grade in 587 instances (91.29 per ct.). This figure probably represents as high a percentage of agreement as can be obtained even under the very best conditions.

6. The results secured from microscopic examination of the milk were found to be more useful than plate counts in interpreting the cause or causes of excessively high counts in the milk. Thus a record of the samples in which the predominant flora consisted of the long-chain streptococci frequent in udder infections, showed that one-fifth of all of the milk delivered at these milk stations which contained bacteria in excess of 1,000,000 per c. c. contained large numbers of streptococci. So far as was determined, all of the milk of this type was originally infected from the udders of some one or more cows in a herd. The conditions observed made it probable that most of the remaining high counts (except where the milk was not properly cooled) were caused by the improper care given cans and milking machines.

7. The simplicity and relative inexpensiveness of the microscopic examination of milk as well as the fact that the microscopic preparations are permanent, gives this technique many advantages over the plating method for commercial work.

INTRODUCTION.

Three years ago the Station published accounts[1] of the results obtained by using a microscopic method of counting the bacteria and cells in milk. At the same time the counts of bacteria were compared with those obtained by the more commonly used agar-plate method. Soon after those bulletins were published plans were made to test the value of the microscopic technique under practical conditions and on a sufficient scale to show its merits and demerits. This experimental work has been in progress during three years.

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[1] Brew, James D. A comparison of the microscopical and the plate method of counting bacteria in milk. N. Y. Agr. Exp. Sta., Bul. 373. 1914.

Breed, Robert S. Cells in milk derived from the udder. N. Y. Agr. Exp. Sta., Bul. 380. 1914.

PLAN OF WORK.

The tests which have been made consist in part of the examination of 2440 samples of market milk at the Hobart bacteriological laboratory conducted by the Lederle Laboratories of New York City for the Sheffield Farms-Slawson, Decker Co. At this place, thousands of agar-plate counts are being made yearly from samples of milk delivered by dairymen to four milk stations operated by this company. Thru the courtesy of Mr. Loton Horton and Mr. William Sheffield of the S. F.-S. D. Co., and Dr. H. D. Pease of the Lederle Laboratories, permission was secured to use the results obtained from these analyses for comparison with the results obtained from grading the milk by microscopic examination.

The results having demonstrated that it is possible to grade milk by microscopic examination in at least as fair a way as by the agar-plate method, cooperative work was then planned with the two milk companies (White Springs Farm Dairy Co. and Geneva Milk Co.) which deliver the larger part of the milk sold in the City of Geneva. In these further tests a sample was taken at least once a week during a period of 26 months from each 40-quart can of milk received by these companies. Microscopic examinations of each sample have been made and the dairymen have been paid during the latter 24 months on the basis of the ratings given.

The cooperation of the men in charge of the two milk stations and that of many of the farmers has been genuine from the beginning of the work. At the end of the first year, and at our request, the dairymen consented to the use of our records by the local board of health. Since that time the work has been carried on in cooperation with this board.

Among the individual men whose help in the work have been the most useful are: Mr. Murray Means, Mr. Gilbert Hubbard and Mr. William Cosgrove, the men who are or who have been managers of the two milk plants during the course of the experiment; Mr. F. E. Rupert, Mr. Charles Rose, and Mr. E. C. Fisher, the officers of the local Dairymen's League, who represent the dairymen delivering milk at the two stations; and Dr. C. D. McCarthy, who as the local health officer, has effectively put into force such control measures as were necessary for the success of the work.

In the work done at Geneva, 11,851 samples of milk have been examined and graded " good," " medium " or " poor " on the basis of the number of bacteria present. The task of collecting and grading has all been done by the junior author (Bw.) except during a period of six weeks in July and August, 1915, and two weeks in May, 1916. During these vacation periods the work was done by W. D. Dotterrer, formerly student assistant in bacteriology.

The technique used in the work was an adaptation of that originally described.[2] The modification have been described in detail in Technical Bulletin 49,[3] and need not be repeated here.

DISCUSSION OF THE RESULTS SECURED AT HOBART, N. Y.

The analyses made by the microscopic method at the Hobart Laboratory were preliminary in character and the technique used was less satisfactory in several respects than that finally devised. The results obtained from the analyses are therefore less accurate than those obtained in the later work done at Geneva and will not be given in detail. The gradings given after microscopic examination of the milk by one of us (Bw.) were compared with the results obtained by Dr. H. V. Tvedt (at that time assistant for the Lederle Laboratories) who used an agar-plating method closely similar to that recommended in the new edition of the Standard Methods of Milk Analysis.[4]

The samples of milk were taken weekly from individual 40-quart cans as delivered by the dairymen to the milk stations. Usually a single sample was taken for each dairyman from a can of milk drawn from 14 to 16 hours previously. At the three stations operated as Grade A, pasteurizing plants for supplying milk to New York City (Bloomville, South Kortright and Cobleskill), the farmers were paid a premium for delivering milk with an agar-plate count of less than 60,000 per c. c. in the winter time and less than 100,000 per

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[2] Breed, Robert S. The determination of the number of bacteria in milk by direct microscopical examination. *Centbl. Bakt.*, Abt. II, **30:** 337-340. 1911.

[3] Breed, Robert S. and Brew, James D. Counting bacteria by means of the microscope. N. Y. Agr. Exp. Sta., Tech. Bul. 49. 1916.

[4] Standard methods of bacteriological analysis of milk. *Amer. Jour. Pub. Health,* **6**:1315-1325. 1916.

c. c. in the summer time. The station at Hobart was operated as a butter factory and the milk was sampled only at irregular intervals and for special reasons. No premiums were paid at the latter plant for milk with a low bacteria count. All samples were iced as soon as taken and sent to the laboratory at Hobart as quickly as possible. In the case of the samples from Bloomville and South Kortright this caused a delay of 12-14 hours before plating could be completed, while the samples from Cobleskill could not be plated until 26-30 hours had elapsed. Ordinary nutrient agar was used for plating and two plates were prepared from each sample, one from a dilution of 1:100 and the other from a dilution of 1:1000. Plates were incubated for five days at a temperature of 20-24° C.

During February, March and April, 1914, 1504 samples of milk from the three Grade A stations were examined by both the agar-plate and the microscopic method. No attempt was made, in making the microscopic examination, to determine the exact number of bacteria present, but the milk was graded merely as A or B milk according as it was thought it would give a plate count under or over 60,000 per c. c. The ratings given were found to agree with those given by the agar plate method in 1339 instances (89.03 per ct.). In 79 cases where the two gradings did not agree, it was because more than 60,000 colonies appeared per c. c. on the plates while the microscopic grading was A. In four of these cases the plate count even exceeded 200,000 per c. c.

In 86 instances where bacteria were found abundant by microscopic examination and it was concluded that the plate count would exceed 60,000 per c. c., the actual plate counts were less than this figure, a number of them being even lower than 10,000 per c. c. In 10 of the 86 samples bacteria were very abundant in the milk, three of the ten showing a predominant long-chain streptococcus flora.

During the following July, 426 additional samples from the same stations were examined and graded by both methods. The border line used in separating the A and B milk at this time in the year as already explained, was a plate count of 100,000 per c. c. The ratings given by the two methods agreed in 362 cases (84.95 per ct.). In 21 cases an A grade was given milk after microscopic examination which showed a plate count in excess of 100,000 per c. c. In 43 cases the microscopic grading given was B while the plate count proved to be less than 100,000 per c. c.

Many of the discrepancies noted in these gradings occurred in the case of samples where the agar-plate count was approximately the same as the number selected as the border line between the two grades so that the error in grading really falls within what might properly be termed a reasonable experimental error; but there were also occasional very wide discrepancies.

One thing which became evident as the work progressed was that it was impossible to get a perfect agreement between the results obtained by the two methods of grading because the counts made by the two methods were not exactly comparable. The agar-plate count was a count of the colonies on the plates. Since these arose from such isolated bacteria or groups of bacteria as were present in the dilution waters and were capable of growth on the plates, this count did not represent either the total number of bacteria present or the number of isolated bacteria and groups originally present in the milk. The microscopic count, on the other hand, represented either the number of isolated bacteria and clumps originally present in the milk, or the total number of bacteria; but could not be made in such a way as to correspond with the plate count. Under these conditions it was impossible to secure perfect agreement in results.

The fact that the more severe ratings were sometimes found by the agar-plate method and sometimes by microscopic examination, caused the one type of discrepancy in grading to offset the other in such a way that there was a very close agreement in the total number of samples graded A or B. Thus out of 1504 samples examined during the winter and spring months from the grade A plants, 1280 were graded A by the plate method and 1270 were graded A by the microscopic method (see summary in Table I). Out of 426 samples graded during July, 234 were graded A by the plate method and 212 were graded A by the microscopic method. This is a surprisingly close agreement and is probably as good as, if not better than, could have been obtained if duplicate analyses had been made of these samples by two analysts using the plate method.

For the sake of comparison later the number of samples graded A and B at each plant has been recorded in Table I. From this it appears that the dairymen met the 60,000 requirement during the winter months better than they did the 100,000 requirement in the summer. The Cobleskill plant continuously received milk with a somewhat lower germ content than did the other plants. The best percentage of A milk recorded in this series of analyses was that for the Cobleskill plant during February, March and April when 87.89 per ct. of the samples gave a count less than 60,000 per c. c. (12.11 per ct. over 60,000).

TABLE I.— QUALITY OF MILK DELIVERED AT FOUR MILK STATIONS AS DETERMINED BY THE PLATE AND THE MICROSCOPIC METHODS.

STATION.	Method used.	NO. SAMPLES GRADED:		Per ct. A.	Notes.
		A.	B.		
Cobleskill Grade A station.	Microscopic...	536	75	86.01	Samples taken during February, March and April, 1914.
	Agar plate....	*545	66	87.89	
	Microscopic...	126	59	68.11	Samples taken during July, 1914.
	Agar plate....	†122	63	74.05	
Bloomville Grade A station.	Microscopic...	540	95	82.41	Samples taken during February, March and April, 1914.
	Agar plate....	*537	98	81.46	
	Microscopic...	51	111	30.86	Samples taken during July, 1914.
	Agar plate....	†52	110	31.48	
South Kortright Grade A station.	Microscopic...	194	64	67.02	Samples taken during February, March and April, 1914.
	Agar plate....	*198	60	69.10	
	Microscopic...	35	44	44.30	Samples taken during July, 1914.
	Agar plate....	†45	34	56.96	
Summary of Grade A stations.	Microscopic...	1,270	234	81.58	Samples taken during February, March and April, 1914.
	Agar plate....	*1,280	224	82.50	
	Microscopic...	212	214	49.76	Samples taken during July, 1914.
	Agar plate....	†234	192	54.93	
Hobart butter factory.	Microscopic...	269	241	52.74	Samples taken during February and March, 1914.
	Agar plate....	*35	36	49.29	

* Samples were graded " A " if the agar plate count was under 60,000 per c. c. during the cold months.

† Samples were graded " A " if the agar plate count was under 100,000 per c. c. during the summer months.

The summary shows that out of the 1504 samples examined during February, March and April, 82.5 per ct. gave a plate count under 60,000 per c. c. (17.5 per ct. over 60,000). This percentage of samples with a low germ content fell greatly during July, when out of a total of 426 samples only 54.93 per ct. gave a count under 100,000 (45.07

per ct. over 100,000). Essentially the same percentages were found when the grading was done by the microscopic method. The fact that less high-count milk was brought to the Grade A pasteurizing plants than to the butter factory indicates the value of the type of control work which was carried out.

DISCUSSION OF RESULTS SECURED AT GENEVA.

All of the work done at Geneva has been done with the improved technique described in Technical Bulletin 49, and under more carefully controlled conditions. As a result of the findings at Hobart it was felt that sole reliance could be placed upon the microscopic count without doing anyone injustice.

Procedure followed.— Samples taken, by means of sterile aluminum tubes,[5] from individual 40-quart cans of morning and night milk before pasteurization, were placed in sterile, cotton-plugged test tubes. The samples were ordinarily secured between 6.30 and 8.30 A. M. as the milk was delivered at the receiving platform, the number of samples taken being approximately the same as the number of cans of milk received. Records have been kept which show the approximate age of the milk sampled (2-4 hours for the morning milk and 14-16 hours for the night milk). Since January 26, 1916, temperature records have been kept as well. The samples have been collected at least once weekly; and during two periods of seven days each, every can of milk delivered at one of the milk stations was examined.

The samples were iced except in cold weather and brought immediately to the Station laboratory about one mile distant from the milk stations. The milk smears were prepared at once. Unless interruptions occurred, the preparation, examination and recording of results were always completed before noon. Frequent timing of the various operations showed that 50-60 samples could be readily smeared, stained and examined in four to five hours. As the gradings were made they were entered on the permanent record sheets and also on the blanks which were mailed to the dairymen. The reports were sent out on an afternoon mail and delivered to the dairymen on the following morning. Where anything of especial interest was found the report was telephoned to the dairyman concerned.

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[5] Schroeder, M. C. A study of the bacteriological and sanitary condition of the milk supply of New York City. *Jour. Inf. Dis.*, 11:1-20. 1912.

BLANK FORM USED IN MAILING DAILY REPORTS TO DAIRYMEN.

DEAR SIR:

Samples taken from the cans of milk which you delivered on ....analyzed as follows:

NIGHT MILK		MORNING MILK	
Can 1........	Can 4........	Can 1........	Can 4........
Can 2........	Can 5........	Can 2........	Can 5........
Can 3........	Can 6........	Can 3........	Can 6........

Average rating............

Yours truly,

Asst. Bacteriologist.

BLANK FORM USED IN MAILING MONTHLY REPORT TO DAIRYMEN.

DEAR SIR:

The following is a report of the analyses made on the milk delivered by you during the month of.................

Total number of cans analyzed............

Number of cans rating as
Good..........
Medium........
Poor..........

Your monthly rating is...................

Yours truly,

Asst. Bacteriologist.

As this milk was sold under the regulations of the State Sanitary Code[6] established by the State Department of Health, an attempt was made to use standards comparable to those of the Code. According to these standards all milk to be pasteurized as Grade A must show a plate count less than 200,000 per c. c.; and all milk to be pasteurized as Grade B must show a plate count less than 1,500,000 per c. c. Experience had shown that these limits were approximately equivalent to microscopic counts of individual bacteria of 1,000,000 and 5,000,000 per c. c. However, since it proved difficult to work with limits as close together as these, and since relatively little milk was found with counts higher than 5,000,000, it was decided to use 1,000,000 and 10,000,000 as the two limits and to designate the three grades of milk thus separated as "good," "medium" and "poor" in order to avoid confusion with the A, B and C grades established by the Code.

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[6] The Sanitary Code established by the Public Health Council of the State of New York. Chapter III. Milk and cream. 1914.

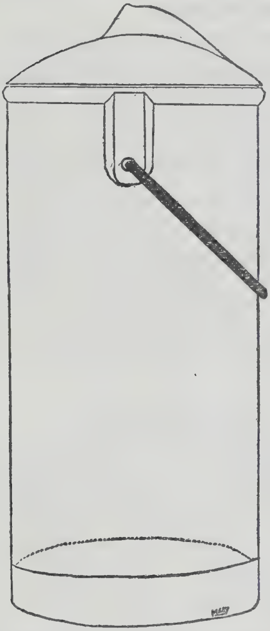
Fig. 5.—Type of Wide-mouthed Specimen Jars Used in Staining Slides.

No attempt was made to secure exact counts of the number of bacteria present except in the case of occasional samples whose grade was doubtful after an examination of 30 microscopic fields (microscope adjusted so that $\frac{1}{300,000}$ cubic centimeter of milk was visible in each field). Some of the same samples have been used for making the comparative plate and microscopic counts discussed in Bulletin No. 439.[7]

Single smears were prepared from each sample and these were arranged in two rows of eight each on special glass slides, 2 x 4½ inches in size, cut from window glass.[8] The xylol or gasolene, alcohol, and stain were kept in wide-mouthed specimen jars, holding 350 c. c., of the type shown in Fig. 5. All of the glass plates on which the smears were mounted have been filed away in an ordinary card catalog case with 2-inch library cards and guides, for notes and for separating the plates. In this way a record has been kept for each farm which has thus far proved both permanent and useful.

The preservation of this record, which readily permits the checking up of any part or all of the past work by a second person, has a constant tendency to prevent careless work in grading. Likewise, whenever a dairyman has doubted the fairness of the report upon his milk, or (as has more frequently happened) has wished to know why the milk which he delivered was considered better or worse than that brought by his neighbors, it has been very easy to place our evidence before him in a way which he could understand. The simplicity of the technique and the quickness with which preparations can be made is such that it frequently happened that preparations were made and examined while some interested dairyman was watching. The process of examination is so direct that anyone can see that there is little chance for deception or mistake.

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[7] Brew, James D., and Dotterrer, W. D. The number of bacteria in milk. N. Y. Agr. Exp. Sta., Bul. 439. 1917.

[8] Obtainable from any hardware dealer.

Very early in the course of the examination of samples taken from individual cans of milk it was noticed that occasional samples were found which showed a predominant flora of long-chain streptococci usually associated with body cells and ordinarily occurring in but one can of night milk and one can of morning milk from the same farm. The striking nature of these characteristics and the almost self-evident explanation of the source of the bacteria in inflamed udders led to the keeping of a record of these samples. No other qualitative records have been kept, altho it is probable that other valuable records could have been made if more information had been at hand concerning the nature and source of the various types of bacteria seen.

A difficulty arose in computing monthly averages because of the fact that not all of the cans of milk delivered from any one farm were of the same quality. Thus, for example, it would be found that of the cans examined Farmer A had delivered 16 cans of " good " milk, 3 cans of " medium " milk and 1 can of " poor " milk. Should his grade for the month be "good" or "medium"? Or Farmer B showed a record of 15 cans of "good" milk and 1 can of "poor" milk. Manifestly even the latter record is not as good as that of a man who delivered milk all of which was graded " good." After some study, the following entirely satisfactory rules were adopted.

RULES FOR COMPUTING DAILY AND MONTHLY RATINGS.

1. Where a man brings not more than 25 per ct. of his milk of " medium " grade he may still be given a " good " rating.

2. Where a man brings not more than 25 per ct. of his milk of " poor " grade he may still be given a " medium " rating.

3. In computing percentages, one can of " medium " milk shall be counted as offsetting 3 cans of " good " milk; likewise, 1 can of " poor " milk as offsetting 3 cans of " medium " milk.

Using the above rules Farmer A would receive a rating of " medium " for the month as he had delivered but 16 cans of " good " milk as opposed to the equivalent of 6 cans of " medium " (the one can of " poor " being counted the same as three cans of " medium "). Six being greater than one-fourth of 22, the average rating would be " medium." Farmer B, on the other hand, would be given a rating of " good " as he had delivered 15 cans of " good " milk as opposed to an equivalent of three cans of " medium " and three is less than one-fourth of eighteen.

The same rules have been followed in computing yearly percentage ratings and also those given in Table II (p. 256–7). Thus the percentage of " good " milk given for the man placed at the head of the list in Table II is 98.93 per ct. altho he only brought one can of milk in all of the twenty-six months which was given a rating lower than " good." As his rating on this can was " poor," the percentage given was computed as if he had delivered 274 cans of milk instead of 272, three of which were " medium." This makes his percentage very little better than that of his neighbor who had 210 cans of milk examined in the same period, three of which were " medium " in grade.

Underlying these rules is the idea that it will take at least three cans of a better grade milk to so dilute one can of a poorer grade milk as to bring the number of bacteria within the number required for the better grade. Theoretically, this gives the advantage to the man bringing the poorer grade but actually, since relatively little of the " medium " and " poor " milk was brought, it was found to work out very fairly. Moreover it gave such an apparent advantage to the dairymen that it was always easy to meet any objections on their part. Thus it is self-evident that if a can of " medium " milk contained any number of bacteria in excess of 4,000,000 per c. c. and less than 10,000,000 it would give a count in excess of 1,000,000 per c. c. if mixed with three cans of the very best quality milk. Or if any can of " poor " milk contained more than 10,000,000 per c. c. it would give a count in excess of 1,000,000 if mixed with nine cans of the finest quality milk.

Results secured.— A tabulated summary of the results secured during twenty-six months is given in Table II. It appears from this summary that 11,851 cans of milk have been examined, 10,166 (85.78 per ct.) of which were found to contain less than 1,000,000 individual bacteria per c. c., and were rated as " good," 1554 (13.11 per ct.) of which had between 1,000,000 and 10,000,000 individual

TABLE II.— SUMMARY WHICH SHOWS THE GRADE OF THE MILK

No.	1915.											1916.							
	Feb.	Mar.	Apr.	May.	Je.	Ju.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Je.	Ju.	Aug.
1	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
2	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
3	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
4	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	G	G	G	G	G
6	G	G	G	G	G	G	G	G	G	G	G	G	G	M	G	G	G	G	G
7	G	G	..	..	..	..	G	G	G	G	G	G	G	G	..	..	..	..	..
8	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
9	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
10	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
11	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
12	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
13	G	G	G	G	G	G	G	G	..	..	..	..	..	..	..	..	..	..	..
14	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	G	M	G	G
15	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	M	G	G	G
16	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
17	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	M	G
18	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
19	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	M	G
20	G	G	M	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
21	G	G	G	G	G	G	M	M	G	G	G	G	G	G	G	G	G	M	G
22	G	G	G	G	G	G	G	G	G	G	G	M	G	G	G	G	G	G	G
23	G	G	G	G	M	G	G	M	G	G	G	G	G	G	G	G	G	G	G
24	G	G	G	G	G	G	M	G	G	G	G	G	G	G	G	G	G	G	G
25	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	G	G	G
26	G	G	G	G	G	G	M	M	G	G	G	G	G	G	G	G	G	G	G
27	G	M	G	G	G	G	M	G	G	G	G	G	G	G	G	G	G	G	G
28	..	..	..	..	G	M	G	M	G	G	G	G	G	G	G	G	M	G	G
29	G	G	M	G	G	G	G	G	G	G	G	M	M	G	G	G	G	G	G
30	..	..	..	..	..	..	..	..	..	..	..	..	..	..	G	M	G	M	G
31	G	G	G	G	M	M	G	G	G	G	G	G	G	G	G	G	M	M	G
32	G	G	M	G	G	M	M	..	..	..	..	..	..	..	..	..	..	..	..
33	G	G	G	G	G	G	G	G	M	G	G	G	G	G	G	M	G	M	G
34	G	G	G	G	G	G	G	M	G	G	M	G	G	G	M	G	M	M	G
35	..	..	..	..	..	..	..	..	..	..	..	..	..	..	G	G	G	G	G
36	G	G	G	G	G	M	G	M	G	G	G	G	G	M	G	G	G	M	G
37	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	M	M	M	G
38	G	G	M	G	G	M	M	M	M	G	G	G	G	G	G	G	M	M	M
39	M	M	G	G	G	G	M	M	M	G	G	G	G	G	G	G	G	M	G
40	G	G	M	G	G	M	M	M	G	G	M	M	M	M	M	M	M	M	G
41	G	G	M	G	G	G	M	M	M	M	G	G	M	M	M	M	G	M	M
42	M	M	G	G	G	M	M	M	G	M	M	M	G	M	G	G	G	M	G
43	G	G	M	M	M	M	M	M	G	M	G	M	G	M	M	G	M	G	G
44	..	..	..	..	..	..	..	G	M	M	M	..	..	..	..	..	..	..	..
45	Dairyman No. 45 did not begin to deliver until Feb., 1917, and so has not been included																		
46	Dairyman No. 46 did not begin to deliver until Mar., 1917, and so has not been included																		

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* In computing the percentages which were to determine the position of each dairyman in this

Note.— 85.78 per ct. of all the milk was rated " good," 13.11 per ct. " medium " and 1.11 per ct.

DELIVERED BY FORTY-SIX PRODUCERS TO TWO MILK COMPANIES.

1916.				1917.			Number of cans graded.	Number of Cans Graded.			Percentage rating. *	Dairy Score.		No.
Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.		Good.	Medium.	Poor.		1915.	1916.	
G	G	G	G	G	G	G	272	271	......	1	98.93	65.5	65.5	1
G	G	G	G	G	G	G	210	207	3	....	98.51	62.5	61.5	2
G	G	G	G	G	G	G	243	239	4	....	98.35	52.5	....	3
G	G	G	G	G	G	G	256	251	5	....	98.05	60.0	69.0	4
G	G	G	G	G	G	G	92	90	2	....	97.83	....	49.5	5
G	G	G	G	G	G	G	221	217	3	1	97.31	53.5	56.5	6
..	..	..	..	..	..	..	73	71	2	....	97.26	54.0	....	7
G	G	G	G	G	G	G	326	319	6	1	97.26	78.0	84.0	8
G	G	G	G	G	G	G	258	249	9	....	96.51	70.0	69.5	9
..	..	G	G	G	G	G	23	22	1	....	95.65	....	34.5	10
G	G	G	G	G	G	G	234	223	11	....	95.30	79.5	75.0	11
G	G	G	G	G	G	G	232	221	11	....	95.26	66.0	64.5	12
..	..	..	...	..	..	..	67	63	4	....	94.03	....	....	13
G	G	G	G	G	G	G	66	62	4	....	93.94	....	63.0	14
G	G	G	G	G	G	G	421	391	30	....	92.87	70.0	60.0	15
G	G	G	G	G	G	G	306	283	23	....	92.48	71.5	72.0	16
G	G	G	G	G	G	G	517	478	39	....	92.46	88.0	87.0	17
G	G	G	G	G	G	G	234	218	16	....	92.31	61.5	59.5	18
G	G	G	..	..	..	..	38	35	3	....	92.10	....	....	19
G	G	G	G	G	G	G	257	237	19	1	91.51	48.5	42.0	20
G	G	G	G	G	G	G	631	579	51	1	91.47	89.0	83.5	21
G	G	G	G	G	G	G	234	216	16	2	90.76	69.0	70.0	22
G	G	G	G	G	G	G	248	225	22	1	90.00	64.5	66.5	23
G	G	G	G	G	G	G	250	226	23	1	89.68	53.5	54.5	24
G	G	G	G	G	G	G	164	148	15	1	89.16	....	96.0	25
G	G	G	G	G	G	G	296	271	21	4	89.11	70.0	70.0	26
G	G	G	G	G	G	G	299	268	30	1	89.04	52.5	65.0	27
G	G	G	G	G	G	G	152	133	18	1	86.43	67.0	64.5	28
G	G	G	G	G	G	G	526	459	62	5	85.63	46.0	64.0	29
G	G	G	G	G	G	G	76	65	11	....	85.52	69.5	61.0	30
G	G	G	G	G	G	G	205	177	25	3	83.89	....	46.5	31
..	..	..	..	..	..	..	64	52	12	....	81.25	....	....	32
G	G	G	G	G	M	G	456	375	78	3	81.17	50.5	50.5	33
G	G	G	G	G	G	G	887	724	158	5	80.71	62.5	60.0	34
G	G	P	..	..	..	..	60	50	9	1	80.65	....	....	35
M	G	G	G	G	G	G	223	187	31	5	80.25	45.5	55.5	36
G	G	G	G	G	G	G	61	49	11	1	77.78	....	58.5	37
M	G	G	G	G	G	G	271	215	42	14	71.90	60.5	60.0	38
G	G	G	M	M	M	G	278	214	52	12	70.79	60.0	63.5	39
G	G	G	M	G	G	G	401	276	118	7	66.51	76.5	60.5	40
M	M	G	G	G	M	G	844	548	295	1	64.77	53.5	68.5	41
G	G	M	M	M	M	..	414	289	107	18	64.22	73.5	....	42
M	M	M	G	G	G	M	370	216	124	30	50.23	53.5	....	43
..	..	..	..	..	..	..	68	33	26	9	37.07	....	....	44
				Totals......			11,824	10,142	1,552	130	.....	....	....	..
in above list......					G	G	24	21	2	1	80.77	....	70.0	45
in above list......					..	G	3	3	......	....	100.00	....	58.5	46
				Totals......			.11,851	10,166	1,554	131	.....	....	....	

list, each can of " poor " milk was considered as equivalent to three cans of " medium " milk.
" poor."

bacteria per c. c., while only 131 (1.11 per ct.) cans were found in which the number of bacteria were in excess of 10,000,000 per c. c.

It is interesting to compare the bacterial quality of this milk, which represented the larger part of that supplied to the City of Geneva, with the quality of milk delivered by dairymen to similar milk plants in other parts of the State. The most extensive records available for comparison, so far as the authors of this bulletin are aware, are those reported by Schroeder.[9] She gives the results of the analyses of 20,334 samples of milk taken at 432 creameries controlled by 120 milk dealers delivering milk in New York City. The analyses were made by a slightly modified form of the agar-plate method officially recognized at the present time; but the results may be compared with those obtained by the official method.

Out of this large number of samples but 17.41 per ct. were found to give a count in excess of 100,000 per c. c. The Geneva supply shows conditions very similar to these, as but 14.22 per ct. of the samples showed more than 1,000,000 bacteria per c. c. (approximately the same as an official count of 200,000 per c. c.).

A comparison of the results secured at Geneva with those secured under similar conditions at Hobart (See Table I, p. 250) indicates, however, that the milk received at the three Grade A plants there studied was in better shape, so far as the total germ content was concerned, than that received at the Geneva plants. This was particularly true of the best of these plants which was located at Cobleskill. While the comparison cannot be made as directly between the Geneva milk and that received at the Hobart butter factory the large percentage (about 50 per ct.) of milk received at the Hobart factory which showed a plate count in excess of 60,000 indicates that the milk was received in poorer condition than at the Geneva plants.

It is not the purpose of the present bulletin to discuss the effect of paying for the milk on the basis of the number of bacteria present, nor to discuss the general question of milk control. Such discussion is more properly left until the experiment has been continued for a longer period of time. However several points are worthy of mention because of their general interest. It will be noted, for instance, that the relationship between the number of " medium " ratings and the summer season is not as marked as might have been expected from the results secured at Hobart (see p. 249). The fewest " medium " ratings were found in May, 1915, and March, 1917, when but one farmer received this rating. The next lowest months were August, 1916, and January, 1917, when but two " mediums " were found. The highest number of " mediums " was found in July, 1916, when fourteen men were found to have brought milk of this grade. The next highest was in September, 1915, when twelve " mediums " were found.

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[9] See footnote 5.

Table II (second and third columns from the right) shows the dairy scores of the farms as obtained by the Dairy Inspector of the City of Geneva for each of the two years using the dairy score card authorized by the State Board of Health. A comparison of these with the amount of germ life in the milk received shows again [10] how little correlation there is between the bacterial quality of the milk received from a farm and those dairy conditions which are ordinarily given great emphasis in dairy inspection work.

There has been a marked difference in the bacterial quality of the milk received at the plants of the two companies. This is evident from the fact that of the 6665 cans of milk examined at the one company 5396 (80.96 per ct.) graded " good," 1177 (17.66 per ct.) graded " medium " and 92 (1.38 per ct.) graded " poor;" while at the other company out of 5186 cans examined, 4770 (91.98 per ct.) graded " good," 377 (7.27 per ct.) graded " medium " and 39 (0.75 per ct.) graded " poor."

The reason for this difference has been sought but cannot be stated with certainty because of the number of factors involved. Several things indicate that one of the reasons, but not the sole reason (p. 266), is because the cans were not steamed sufficiently and were not properly cared for after steaming at the station which had the greater amount of " medium " and " poor " milk. It is at least noteworthy that a marked decrease in the amount of milk with a high germ content took place after August 1, 1916, coinciding with a simple change in the procedure of handling the cans.

Previous to this date, the cans, after washing, rinsing with a jet of water, and steaming for five to ten seconds, had been allowed to stand upright, thus permitting the steam to condense in them. The covers were then put on and the cans placed in a protected place for twenty-four hours before they were returned to the farms. As shown by Prucha and Weeter,[11] cans handled in this way usually contain millions and frequently billions of bacteria. After August 1, 1916, the procedure was modified by inverting the cans and leaving the covers off. While this did not give a can that was entirely dry, it caused a marked improvement in their condition.

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[10] Compare also Brew, James D. Milk quality as determined by present dairy score cards. N. Y. Agr. Exp. Sta., Bul. 398. 1915.

During April and May, 1917, some special tests were made in the case of dairymen who still continued to have trouble with " medium " and " poor " milk. These revealed the fact that occasional cans from this plant still contained millions or even billions of bacteria which could be dislodged upon rinsing with sterile water. The procedure of washing the cans was again improved so that each can received a steaming of twenty to thirty seconds and was so handled that it was thoroly dry immediately afterward. Bacteriological tests showed them to be in good condition. A still further reduction in the amount of " medium " and " poor " milk occurred, but, because of other changes which took place at the same time, the causal relationship between the two could not be established.

It has been generally recognized [12] that milking machines are a common source of high bacterial counts unless they are properly cared for. The records given in Table II give information of value in estimating the relative amount of contamination from this source. It has been noted in the course of the work that in case milk containing a sufficient number of bacteria to cause it to be rated " medium" or "poor" was found on farms where milking machines were in use, it usually happened that all of the cans of milk were seeded with bacteria in approximately the same numbers. This effect was especially noticeable where thoro cooling of the milk had stopped the growth of the bacteria as soon as milking was completed. Usually such milk contained enough bacteria to cause it to be rated " medium " but not enough to cause it to be rated " poor." The bacteria present were in groups of variable sizes, including some relatively large groups, and were of miscellaneous types.

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[11] Prucha, M. J., Weeter, H. M., and Chambers, W. H. Germ content of milk as affected by the utensils. Ill. Agr. Exp. Sta., Bul. 205. 1918.

[12] Harding, H. A., Wilson, J. K., and Smith, G. A. Milking machines: Effect of method of handling on the germ content of the milk. N. Y. Agr. Exp. Sta., Bul. 317. 1909.

A brief record of the conditions on the six farms where machine milking was practiced during all or part of the twenty-six months will show the relation between the use of machine milkers and high germ counts. Two different types of machines were in use on these farms and a third type has been under observation on other farms. However, since both good and poor records have been made with each type of machine, the name of the machine is not given.

Dairyman 17.— Installed a milker about June 1, 1915. During the four months immediately preceding the installation of the milker, 38 cans of milk were examined from this farm, 36 of which graded " good " and two " medium." During the same four months of 1916, 43 cans of milk were examined, four of which graded " medium." Thirty-six cans were examined during the same period of 1917, three of which graded " medium." The milking was usually done by the owner of the herd and the care given the machine was good. On August 22, 1916, a visit was made to this farm and five liters of sterile water were " milked " thru one unit from an artificial udder.[13] The plate count from this water showed 1,050 colonies per c. c. A second " milking " of sterile water thru the same unit gave water which showed a germ content of 1,190 per c. c. These records indicate that the bacterial quality of the milk at this place was as good after the installation of the milker as before.

Dairyman 21.— Was using a machine milker when the control work was started, but discontinued its use after June 1, 1916. During the year previous to this date, 327 cans of milk were examined from this place, 294 of which graded " good," 32 graded " medium " and one graded " poor." During the following year, in which hand milking was practiced, 278 cans were examined, 259 of which graded " good " and nineteen " medium." A still greater improvement in the quality of the milk from this place occurred during the summer of 1917, for whereas 32 "medium" and one "poor" can had been received from this farm during the summer of 1915, and nineteen cans of " medium " milk during the summer of 1916, not a can of " medium " milk was received during the summer of 1917. Between 1916 and 1917 ice was provided for cooling and the temperature of the milk received during July, August and September, in 1917, averaged 53.9° F. as compared with an average of 57.9° in 1916. Apparently the original trouble at this place was due to a combination of poor cooling, poorly washed milk cans and possibly also the milking machine.

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[13] Tests made by G. L. A. Ruehle. See Ruehle, G. L. A., Breed, Robert S., and Smith, George A. The milking machine as a factor in the production of sanitary milk. *Amer. Jour. Pub. Health,* 7:840-846. 1917.

Dairyman 33.— Installed a machine milker at about the time the control work was started and continued its use thruout the period. Four hundred and fifty-six cans of milk were examined from this farm, 375 of which graded " good," 78 " medium " and three " poor." On August 22, 1916, a visit was made to this farm and five liters of sterile water were " milked " thru the machine from a metal container. This water developed 195,000 colonies per c. c. on agar after it had passed the teat-cups and head of the machine, and 170,000 colonies per c. c. after being drawn into the pail. A second milking thru the same machine showed the water to be capable of developing 40,000 colonies per c. c. after passing the teat cups and head, and 36,000 per c. c. when taken from the pail. In several instances the trouble from "medium" and "poor" milk at this farm stopped for a time after the teat cups had been given a thoro cleaning.

Dairyman 40.— A milker was in use at the time the inspection was started, but machine milking was discontinued July 1, 1916. During the year previous to this date, 176 cans of milk were examined from this farm, 90 of which were graded as " good," 84 " medium " and two " poor." During the following year, in which the cattle were hand milked, 176 cans were examined, 143 of which were graded " good," 29 were graded " medium " and four were graded " poor." These figures indicate the marked improvement which took place at this farm after the machine was discarded. Samples of milk containing long-chain streptococci were frequently found both before and after the machine was discarded.

Dairyman 41.— A milker has been used on this place thruout the period of inspection work. In several instances, the trouble from " medium " milk cleared up for a time after the milking machines were given a thoro cleaning. Again and again every can of milk tested was seeded with enough bacteria to give the milk a " medium " grade regardless of the excellent cooling of the milk and regardless of the age of the milk. Out of 844 cans of milk examined, only 548 have graded " good " while 295 have graded " medium " and one " poor." On August 18, 1916, a visit was made to this farm and five liters of sterile water " milked " thru one unit as in previous tests. The water showed a plate count of 3,174,000 per c. c. A second milking carried out as before gave water which had a plate count of 2,310,000 per c. c. Samples of milk taken from each of two other units immediately after milking showed plate counts of 1,003,000 and 900,000 per c. c. respectively. These facts make it evident that the machines seeded the milk heavily with bacteria, tho in all probability they were not the sole cause of the high counts as samples containing long-chain streptococci were frequently found.

Dairyman 42.— Used a machine thruout the period of inspection. A visit was made to this farm on August 25, 1916, and five liters of sterile water were " milked " thru one unit as at the other farms. The agar-plate count of this water was 1,188,000 per c. c. A similar test made from a second " milking " thru the same unit showed the water to have a plate count of 743,000 per c. c. A sample of milk taken from another unit, immediately after milking a cow showed a plate count of 239,000 per c. c. These tests showed that the machine was not kept in good condition, but since other conditions at this place were such that they may have affected the germ content of the milk appreciably, it is probable that the machine was only one of several causes of trouble.

These men have used solutions of chloride of lime, or of chloride of lime and brine in which to keep the teat cups and other rubber parts of the machine between milkings.[14] A number of tests have shown, however, that the solutions were rarely maintained at the proper strength. All of the machines were on farms where there were herds of at least twenty cows, and all or practically all of the work was done by hired labor. The only exception to this condition was found in the case of Dairyman 17 where the owner himself did the greater part of the work. It is noteworthy that his record is the best of the six men who used machines.

The influence of milking machines on the germ content of the milk is also seen when comparisons are made with the grade of the milk received from the farms where hand milking was practiced. Three thousand and thirteen cans of milk were examined from the dairies of Dairymen 29, 31, 32, 34, 35, 36, 37, 38, 39, 43 and 44. Hand milking was practiced on all of these farms. Out of this total, 2376 cans graded "good," 552 graded "medium" and 85 graded "poor." If these grades are compared with those given the 3051 cans of milk received from the six farms on which machine milking was practiced (2350 cans "good," 672 cans "medium" and 29 cans "poor"), it will be seen that the milk received from the farms where machine milking was practiced had a germ content similar to that of the milk received from the eleven poorest farms where hand milking was practiced. As a class those men who had milking machines stand near the bottom of the list.

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[14] See Milking machines. N. Y. Agr. Exp. Sta., Circ. 54. 1917.

The conditions suggest, however, that *the reason for this was that the machines were not given proper care*, rather than that the machines themselves were at fault. The production of milk with a low germ content from machine milked cows has never been found to be specially troublesome at the experiment station where proper care is given the machines.

A fact worth noting is that a larger proportion of "poor" cans came from farms where hand milking was practiced. This condition of affairs corresponds with that already noted on page 733 where it was pointed out that milk from farms where milking machines were used, while frequently showing a consistently high germ content, did not ordinarily contain enough bacteria in excess of 1,000,000 per c. c. to exceed the limit of 10,000,000 per c. c. This suggests that the usual causes of the excessively high counts were either the poorly sterilized cans (page 259–260), udder infections (page 265–6) or a combination of these with other conditions.

By chance, all of the dairymen who failed to keep their machines free from bacteria, delivered their milk to one of the two companies. This fact, together with the poor cleansing of the cans undoubtedly explains why the one company received more milk with a high germ content than did the other company.

The records which have been kept of the number of "medium" and "poor" cans of milk in which the organisms present were largely long-chain streptococci have proved very instructive. Only eight of the thirty men who have delivered milk continuously during the twenty-six months have done so without bringing a can of milk of this type. Out of the total number of cans examined (11,851), 345 or 2.91 per ct. have shown a predominant streptococcus flora in excess of 1,000,000 per c. c. The 345 cans amounted to 20.47 per ct. of all the "medium" and "poor" milk. Many other cans of milk were noted where these organisms were present in smaller numbers or where they were not the predominant organism.

The evidence which connects the presence of these organisms with udder infections, while negative and circumstantial in some cases, nevertheless seems conclusive. In each of the nine instances where the farm supplying the milk has been visited and samples taken from each cow in the herd, the offending animal or animals have been found at once. In other cases, where the dairyman has been asked to be on the lookout for garget, the trouble has disappeared from the milk, and later inquiry has established the fact that the milk of some one or more suspected animals was being discarded.

The streptococci almost invariably appeared in a single can of night milk and a single can of morning milk and were frequently associated with body cells. The morning milk occasionally contained noticeably fewer organisms than the night milk, the marked differences in numbers being ordinarily associated with temperatures in the night milk in excess of 60° F. In such cases the most natural explanation was that the growth of the organisms in the milk after it was drawn had caused the differences in numbers. At times, the conditions found have suggested that the utensils were seeded with the streptococci and that the immediate source of the organisms was the utensils; but if this was the case the fact was not established.

The streptococcus-infected milk has come largely from four herds out of the forty-six. In one case more than half of the animals in the herd were, at one time, found to give milk containing streptococci. In many cases the milk containing the streptococci was normal in appearance as drawn and there was no evident inflammation of the udder. In other cases, there was evident inflammation of the udder (mammitis or garget). Instances were also noted where the milk was abnormal in appearance and there was evident garget, yet no streptococci were found in the milk.

Two of the four herds from which the milk containing streptococci was frequently received, were milked by hand and two by machine. The machine milker was discarded at one of the two latter farms partially because of a belief that it has caused garget. Nevertheless so far as the records of the milk examinations show, the garget cleared up at the farm where the machine milking was

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continued just as quickly as at the farm where it was discarded. Both farms occasionally brought milk containing many streptococci during the year that followed.

No systematic studies of the streptococci have been made, and all cocci tending to grow in chains including more than four to six individuals have been included in this group. It is therefore probable that a variety of types or species are included under this term.

A lookout has been maintained for other abundant organisms derived from the udder but none have been detected except in one instance. The organism in this case was a tiny micrococcus which was traced back to the udder of a single animal in a small herd. This animal was giving milk of a normal appearance but was not a profitable animal and was sold before an opportunity was presented to study the matter further.

The failure to find organisms which might be identified with the tubercle organism, or other organisms which have been described as occurring in the udder in large numbers was surprising. This negative result may mean little, however, as small numbers of any of these organisms might have been overlooked, and large numbers may occur but rarely. It is possible, moreover, that a careful identification of the organisms in the microscopic preparations would have permitted the recognition of some of these organisms.

The result of these attempts to recognize the causes of the high-count milk has been to indicate that the three things which have just been discussed (improperly cleaned milk cans, improperly cared for milking machines, and streptococcic udder infections) have caused nearly all of the high germ counts which were due to initial contamination of the milk. This includes nearly all of the milk examined, as conditions favorable to an extensive growth of the organisms in the milk were uncommon. Even the night milk was only twelve to sixteen hours old when examined and in the majority of cases was cooled below 60° F.

Of the 11,851 samples considered 643 were examined by the plate method as well as by the microscopic method.[15] Out of this number 518 samples showed fewer than 1,000,000 bacteria per c. c. by the microscopic method and less than 200,000 colonies per c. c. by the plate method; fifty-eight samples showed more than 1,000,000 and less than 10,000,000 bacteria by the microscopic method and at the same time a plate count in excess of 200,000 and less than 1,500,000 per c. c.; while eleven samples showed more than 10,000,000 bacteria per c. c. and at the same time gave a plate count in excess of 1,500,000 per c. c. In other words, if we accept the limits set as marking the limits between A, B and C milk, there was an agreement in the ratings given these samples in 587 cases out of 643 (91.29 per ct.).

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[15] See footnote 7.

The discrepancies in ratings were grouped as follows: 24 samples of milk which were rated as " good " by the microscopic method were rated as grade B by the plate method; likewise 24 samples of milk which were rated as " medium " by the microscopic method were rated as grade A by the plate method. Eight samples were rated as " medium " by the microscopic method which were classed as grade C by the plate method. The latter type of discrepancies, as already explained (p. 252), was due to the fact that the 10,000,000 limit used in the case of the microscopic method is more lenient than the 1,500,000 limit used in the case of the plate method.

Attention should be called to the fact that the agar-plates used in making these counts were incubated for five days at 21° C. followed by an incubation of two days at 37° C., so that the counts obtained were in all probability higher than they would have been if the plates had been incubated according to the procedure ordinarily used in routine work, namely, two days at 37° C.

Table III gives the results secured during a seven-day period in August, 1915, during which time each can of milk received at one of the two companies was sampled. Table IV gives similar results from samples taken at the same milk station during a seven-day period in February, 1916, while Table V gives a summary of the results in Tables IV and V. Between 33 and 51 samples were taken per day. The work of preparing materials, collecting and examining all of the 574 samples was done by the junior author (Bw.) and did not prove an especially burdensome task. All results were ready soon after noon of the day on which the samples were taken.

The chief purpose in taking these samples was to learn whether a reasonably accurate judgment was secured in regard to the quality of the milk brought by an individual dairyman when samples were taken on one day per week. The results showed that during the

August period there were only four of the seventeen dairymen who brought milk of such quality that all cans graded " good." In the winter time (February), this number was increased so that all but three of the seventeen brought milk, all of which graded " good." In the case of these men it is evident that it made no difference how many samples were taken, as the rating for the week would have been the same regardless of the number of samples taken.

The fact that it is necessary to take samples of both morning and night milk in order to get a fair estimate of the quality of milk brought is shown by the summary (Table V) as well as by many individual instances (Tables III and IV). The night milk being twelve to sixteen hours old naturally more frequently falls into the " medium " and " poor " classes than does the morning milk. Thus in the winter time only three " medium " cans appeared in the morning milk, while there were ten cans of night milk which graded " medium." The contrast between the night and morning milk was still more striking in the summer months where there were forty-one cans of " medium " milk and four cans of " poor " milk in the night milk as contrasted with only eleven cans of " medium " and none of " poor " morning milk. From these findings, it is evident that if samples had been taken from night milk only for use as a basis of payment to the dairymen, the ratings given would have been more severe than were warranted. This injustice would have been worse in the summer months than in the winter time.

The surprisingly great variation in the quality of the milk found in some instances in different cans of milk as delivered by the same dairyman on the same or different days makes it evident that it is no easy matter to sample the milk delivered in such a way as to secure a reasonably accurate grading. The difficulties involved are far greater than those involved in getting an accurate butter-fat test.

It was disappointing to find that because of the highly variable conditions the records secured were neither detailed enough nor extensive enough to permit a satisfactory mathematical analysis of the results. If commercial use is to be made of the bacterial count in the buying and selling of milk, it is highly important that studies be made to determine how many samples should be taken each week in order to secure reasonably accurate results.

Table III.— Grades Given the Individual Cans of Milk Received at a Milk Station during a Seven-day Period in August.

Dairyman No.		Aug. 25.	Aug. 26.	Aug. 27.	Aug. 28.	Aug. 29.	Aug. 30.	Aug. 31.
1	P. M.*	G†	G	GG	G	GG	G	GG
	A. M.	GG	GG	GG	G	GG	GG	GG
3	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
4	P. M.	G	G	G	G	G	G	G
	A. M.	GG	GG	GG	G	GG	GG	GG
6	P. M.	G	G	G	G	M	G	G
	A. M.	G	G	G	G	G	G	G
7	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
9	P. M.	M	G	M	G	M	M	G
	A. M.	GG	G	GG	GG	GG	GG	GG
11	P. M.	G	G	M	M	G	G	G
	A. M.	G	G	G	G	G	G	G
16	P. M.	GM	GM	GG	GG	GG	GG	GG
	A. M.	GG	GG	GG	GG	GG	GG	GG
18	P. M.	G	M	G	G	M	M	M
	A. M.	G	G	G	G	G	G	G
20	P. M.	MM	GG	GG	GG	GG	GG	GG
	A. M.	G	G	G	G	G	G	G
23	P. M.	M	G	G	G	M	M	M
	A. M.	G	G	G	G	G	G	G
24	P. M.	M	M	G	G	M	P	M
	A. M.	G	G	G	M	M	M	M
26	P. M.	MM	PG	MG	G	MG	MG	PG
	A. M.	GG	GG	GG	G	GG	GG	GG
27	P. M.	M	G	G	GG	GG	MM	GG
	A. M.	GG	GM	GM	MM	MM	GG	GG
28	P. M.	M	M	G	G	G	G	M
	A. M.	G	G	G	G	G	G	G
29	P. M.	GGG	GGG	GGG	GGG	MGG	MMMMM	GGGG
	A. M.	GGG	GGG	GGG	GGGG	GGGG	GGGG	GGGG
39	P. M.	P	G	G	G	M	M	G
	A. M.	G	G	G	G	G	M	G

* It was usually possible to recognize which was night milk and which was morning milk from the temperature, as the morning milk was not cooled. It was evident that morning and night milk were mixed in certain cases.

† Each single letter represents a can of milk. G signifies less than 1,000,000 bacteria per c. c., M more than 1,000,000 and less than 10,000,000, and P more than 10,000,000.

TABLE IV.—GRADES GIVEN THE INDIVIDUAL CANS OF MILK RECEIVED AT A MILK STATION DURING A SEVEN-DAY PERIOD IN FEBRUARY.

Dairyman No.		Feb. 16.	Feb. 17.	Feb. 18.	Feb. 19.	Feb. 20.	Feb. 21.	Feb. 22.
1.........	P. M.*	G†	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
3.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
4.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
6.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
7.........	Mixed	G	G	G	G	G	G	G
9.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
11.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
16.........	P. M.	G	G	G	M	G	G	G
	A. M.	G	G	G	......	G	G	G
18.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
20.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
23.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
24.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
26.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
27.........	P. M.	M	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G
28.........	Mixed	G	G	G	G	G	G	G
29.........	P. M.	MM	MM	MG	MM	GG	GG	MG
	A. M.	GG	MM	MG	GG	GG	GG	GG
39.........	P. M.	G	G	G	G	G	G	G
	A. M.	G	G	G	G	G	G	G

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* It was usually possible to recognize which was night milk and which was morning milk from the temperature, as the morning milk was not cooled. It was evident that morning and night milk were mixed in certain cases.

† Each single letter represents a can of milk. G signifies less than 1,000,000 bacteria per c. c., M more than 1,000,000 and less than 10,000,000, and P more than 10,000,000.

TABLE V.— SUMMARY OF GRADES GIVEN IN TABLES III AND IV.

	NUMBER OF CANS GRADED:			Totals.
	Good.	Medium.	Poor.	
1915.				
August 25	36	10	1	47
August 26	40	5	1	46
August 27	44	4	....	48
August 28	41	4	....	45
August 29	39	11	....	50
August 30	36	13	1	50
August 31	45	5	1	51
P. M. †	117	41	4	162
A. M.*	164	11	....	175
Totals	281	52	4	337
1916.				
February 16	31	3	....	34
February 17	30	4	....	34
February 18	32	2	....	34
February 19	30	3	....	33
February 20	34	....	....	34
February 21	34	....	....	34
February 22	33	1	....	33
P. M.†	102	10	....	122
A. M.*	108	3	....	111
Mixed	14	....	....	14
Totals	224	13	....	237

† Milk was approximately 14-16 hrs. old.
* Milk was approximately 2-4 hrs. old.

CONCLUSIONS.

The use of the microscopic method in grading 2,440 samples of market milk according to its bacterial quality at Hobart, N. Y., has shown that this technique can be used successfully under commercial conditions as a means of distinguishing between milks containing few and many bacteria. In view of the possible chances of error present in the case of both the microscopic and the plating methods of grading when used as they were at Hobart, it was gratifying to find as high a percentage of agreement in the grades as 84–89 per ct. There is reason to believe that the results agreed as well as they would have done if the two sets of grading had been made by two different analysts using the officially recognized plate method.[16]

Inasmuch as there is no available standard by which the accuracy of the results obtained by either the plate method or the microscopic method can be tested, it was impossible to determine the relative accuracy of the results obtained by the two methods of grading. However, since the grades given individual samples by each method were found to agree under carefully controlled conditions in 91 per ct. of the cases, it raises the presumption that the grades given the samples by both methods were reasonably accurate.

The more carefully controlled experiment carried out at Geneva for twenty-six months shows that it is entirely feasible and practicable to use the microscopic method for the commercial grading of milk into as many as three grades. The labor involved and the expense of equipment are much less than that required where the plate method is used; while still more important, especially to the producer, is the fact that the results are available within a few hours and the preparations can be preserved indefinitely. The latter fact permits the checking of fraudulent practices in grading in a very satisfactory and simple way. This does not mean that the technique is fool proof. It is capable of being misused either intentionally or unintentionally just as readily as is the Babcock test, a misuse which has caused much dissatisfaction in the dairy industry.

The microscopic examination of the milk was found to be more useful as a means of detecting the cause or causes of excessively high germ counts than was the plate method. This was particularly true in regard to the detection of high counts due to udders infected with streptococci. By its use it was possible to determine the approximate percentage of the high-count milk which was due to this cause. The fact that one-fifth of all the high-count milk was of the streptococcus type was a great surprise and indicates that much greater attention should be given to this source of high counts than is ordinarily given.

In addition to the infected udders of particular animals, there appeared to be two other important sources of the excessively high germ counts: Namely (1) improperly steamed and poorly drained milk cans which stood with covers on in places where the temperatures favored the growth of bacteria, and (2) milking machines which were not taken apart and thoroly cleaned frequently enough and which were not kept in sufficiently strong brine, or brine and chloride of lime. Because of cooling of the night milk and the fact that none of the milk was over sixteen hours old when tested, the growth of bacteria played a smaller part in producing the high counts than is the case where milk stands a longer time before delivery.

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[16] Compare, for example, the results secured by analysts working in four New York City laboratories as given by Conn, H. W. Standards for determining the purity of milk. *Pub. Health Repts.*, 30:2349-2395. 1915.

The preliminary study made in order to learn the proper number of samples necessary to determine the grade of the milk with reasonable accuracy gave results which were instructive. While some of the dairymen brought milk whose quality did not vary greatly in the different cans of milk delivered on any one day, or on consecutive days, other men brought milk whose quality was highly variable. It will therefore be necessary to study more extensive data gathered under a variety of conditions before any satisfactory answer can be given to this question. Enough was learned, however, to show that no fair estimate of the bacterial quality of the milk can be made from samples taken from morning milk only, or from night milk only.

The final result of this work has been such as to fully justify the Committee on Standard Methods of Bacterial Milk Analysis in including a description of the microscopic technique in their final report.[17] Likewise the reports from several of the large milk distributing companies in the State that are using this technique at the present time indicate that they are already finding it useful in their laboratories.

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[17] See footnote 4.

REPORT

OF THE

Department of Botany.

F. C. Stewart, *Botanist.*
W. O. Gloyer, *Associate Botanist.*
M. T. Munn, *Assistant Botanist.*

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II. Neck-rot disease of onions.

REPORT OF THE DEPARTMENT OF BOTANY.

BLACKHEART AND THE AERATION OF POTATOES IN STORAGE.*

F. C. STEWART AND A. J. MIX.

SUMMARY.

Present knowledge of blackheart in potatoes rests upon investigations made by Bartholomew who proved that it may result from exposure of the tubers to a temperature of 38–45° C. for 14–48 hours and is due to changes in the tissues caused by a derangement of the process of respiration.

The accidental discovery that by excluding the air from potatoes blackheart may be produced at temperatures much lower than those employed by Bartholomew led the writers to make experiments with tubers in sealed jars.

From such experiments it was learned that potatoes cannot long endure close confinement. Within a certain length of time, which varies with the temperature and quantity of air available, tubers confined in hermetically sealed jars become moist over a part or the whole of their surface. If the tubers are then exposed to the air the moist surface areas turn brown and the color of the flesh changes first from white to pink then to black (blackheart).

With a volume of air equal to the volume of the tubers a confinement of ten or twelve days is sufficient to produce the symptoms described provided the temperature is around 70° F. At a temperature of 55–60° F. about twenty days are required; and at 40° F. a still longer time — somewhere between twenty-three and forty days.

Tubers in half-full and quarter-full jars behave similarly to those in full jars except that the symptoms are slower in making their appearance.

Tubers confined in sealed jars with less than about ten times their volume of air are unable to do more than barely start sprouts. For normal sprouting about nineteen volumes of air per volume of

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* Reprint of Bulletin No. 436, June, 1917.

tubers are required. Blackheart may be expected to appear whenever the volume of air available to the tubers is less than that required for normal sprouting.

Different tubers of the same lot exhibit marked differences in susceptibility, both to blackheart and to surface discoloration. The cause of this has not been determined. It appears doubtful if the size of the tubers is an important factor.

Upon learning of the injurious effects of insufficient aeration the writers made other experiments with tubers in deep tanks to determine how deeply potatoes may be piled with safety. The data obtained are insufficient for the formulation of definite rules, but it appears that six feet should be considered the maximum depth of piling when potatoes are to be stored for several months at temperatures below 45° F. At temperatures above 50° F. the depth limit should be three feet if the potatoes are to be stored longer than three or four weeks.

Tubers suffering from insufficient aeration thru deep piling behave, in a general way, like tubers in sealed jars. They sprout feebly or not at all, become moist on the surface, discolor externally upon exposure to the air, and are often affected with blackheart internally. The principal difference is in the occurrence of rotten spots caused by fungi and bacteria. This form of injury is more common with potatoes in deep piles than with potatoes in sealed jars.

Blackheart sometimes occurs in potatoes stored out-of-doors in pits and is due, undoubtedly, to insufficient aeration. However, the experiments so far made indicate that the aeration of potatoes in unventilated pits is better than might be supposed and that the ventilation of small pits is unnecessary.

The injury resulting from insufficient aeration is due to the lack of oxygen rather than to the accumulation of carbon dioxid.

Tubers affected with blackheart produced by exposure to high temperature usually appear normal externally, while those affected with blackheart produced by exclusion of the air usually show more or less surface discoloration.

Insufficient aeration during storage does not cause spindling-sprout. It may retard sprouting temporarily, but when, subsequently, the tubers are supplied with air they sprout normally if at all. Tubers severely affected with blackheart are unfit for seed

purposes, but slightly affected tubers may be planted. If tubers are sound and normal in appearance it is unlikely that they have been injured for seed purposes by any storage conditions to which they may have been subjected.

The prevention of blackheart is a shipping problem as well as a storage problem. Blackheart often results from the overheating of potatoes during shipment in stove-heated cars.

BARTHOLOMEW'S WORK ON BLACKHEART.

Blackheart is the name proposed by Bartholomew for an abnormal condition of potato tubers in which the central portion is dark brown or black. Affected tubers may appear normal externally, but when cut open they show an area of dead, black tissue, occupying a large portion of the interior. Frequently, the trouble takes the form of a cavity lined with a thick layer of tough, dead, black tissue.

Our knowledge of the cause and nature of blackheart rests upon the investigations of Bartholomew who has published two papers on the subject.[1] On page 618 of his second paper he says: "That the production of black heart in potatoes is due to changes in the tissues caused by overheating in an atmosphere devoid of sufficient oxygen to meet the demands of the rapidly respiring potatoes can not be questioned." He found blackheart occurring among potatoes shipped long distances in stove-heated refrigerator cars and came to the conclusion that the trouble resulted from overheating in transit. While considering this a common cause he recognized that there are, also, other causes. He says:[2] "No doubt this malady may occur in potatoes at other times than during shipment. Experiments have shown that the only conditions necessary are excessive temperatures and a lack of sufficient oxygen. It is entirely possible and even probable that such conditions may exist where the potatoes have been stored in close warm cellars or in pits covered with manure."

Proof of his statement that overheating is a cause of blackheart is found in the fact that he was able to produce blackheart at will by subjecting tubers to temperatures of 38–45° C. (100–113° F.) for 14–48 hours.

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[1] Bartholomew, E. T. Black heart of potatoes. *Phytopathology*, 3:180–182. 1913.
——— A pathological and physiological study of the black heart of potato tubers. *Centbl. Bakt.* etc. II, 43:609–638. 1915.

[2] *Loc. cit.*, p. 623.

Altho Bartholomew recognized that both the temperature and the oxygen supply are factors in the production of blackheart, and notwithstanding his statement[3] that, "it may be prevented in shipping, or otherwise, by proper ventilation and by keeping the potatoes in a temperature which does not exceed 35° C.", it seems he did not realize that blackheart may occur at temperatures considerably lower than 35° C. provided the supply of oxygen is scant. There is nothing in either of his papers to indicate that he succeeded in producing blackheart at a temperature lower than 38° C.

ORIGIN OF THIS INVESTIGATION.

In the spring of 1914 it was observed that some tubers enclosed for about two weeks in an air-tight glass jar had failed to sprout, and when cut they were found to be affected with blackheart. Since the temperature to which the tubers had been exposed could not have been at any time higher than about 20° C. the blackheart must have come about in a manner somewhat different from that described by Bartholomew.

A few experiments demonstrated that the air supply has an intimate relation to the occurrence of blackheart, and that a considerable volume of air is required for the well-being of potatoes. This led to further experimentation to determine how large a quantity of air is required, and what is the effect of storing potatoes in deep piles in cellars and bins and in unventilated pits and piles out-of-doors.

METHODS AND APPARATUS.

Most of the experiments were made with tubers in wide-mouthed glass museum jars having a capacity of 3500–3700 cubic centimeters (about 3.5 quarts) and provided with tight-fitting, ground-glass stoppers. A few experiments were made in smaller jars of the same kind. The jars were made air-tight by running melted paraffin or sealing-wax around the stoppers. Using tubers weighing from two to four ounces it required about four pounds of potatoes to fill one of the larger jars. In full jars the volume of the air enclosed with the tubers was equal, approximately, to the volume of the tubers. It varied from about 28 to 31 cubic centimeters for each ounce of tubers. By filling the jars only partly full other ratios were obtained.

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[3] *Loc. cit.*, p. 637

For the effect of storing potatoes in deep piles use was made of tall, galvanized-iron cylinders which were open at the top but air-tight on the sides and bottom. The diameter of these cylinders was nine inches. Their hight varied from 80 to 126 inches.

One experiment was made out-of-doors with piles of potatoes which were protected from freezing by covering them with oat-straw and soil according to the usual custom.

Unfortunately, it was impossible to control the temperature except within rather wide limits,— 5 to 10° Fahr. The highest and lowest temperatures employed were about 75 and 35° F. (24 and 2° C.) respectively.

The experiments were conducted during April and May, 1914; from January to May, 1915; and from October, 1915, to May, 1916. A single variety of potato, Sir Walter Raleigh, was used thruout. In all cases, excepting the out-door pit experiment, the tubers were thoroly washed. Only perfectly sound tubers were used and they were dry when put into the jars or tanks.

BEHAVIOR OF TUBERS IN FULL, SEALED JARS AT 70° F.

When jars completely filled with tubers are hermetically sealed and exposed to a temperature of about 70° F. the following results may be expected:

No sprouts will start; and any sprouts which may have been on the tubers at the time of sealing the jars will blacken and die. Otherwise, the appearance of the tubers will be normal until about the tenth day. Then the tubers will commence to show moist areas (" sweat "). This will appear earlier on some tubers than on others. By the fifteenth day the tubers will be moist over their entire surface. If removed from the jar before the appearance of the moist areas the tubers will, usually, show no discoloration externally and, if given a chance, they will sprout normally. If removed after they have begun to sweat the tubers quickly discolor upon exposure to the air. The discoloration is a shallow, brown one which appears over well-defined areas of various extent. It may cover any part or even the whole of the surface. The bud end of the tuber appears to be particularly susceptible. The stem end portion is least susceptible. (Plate IX.) In a general way the discolored areas coincide with the moist areas, but there are numerous exceptions to the rule.

After a few days' exposure to the air the discolored areas become slightly depressed and drops of a brown, sticky exudate appear here and there. Tubers having less than about half of their total surface discolored resist decay for a long time if kept dry, and may even produce sprouts; but tubers more severely affected soon decay. Besides the large discolored areas above described there often occur, also, circular, dead, brown, depressed spots two to five millimeters in diameter. These resemble considerably the spots produced by fumigation with formaldehyde gas as described in Bulletin No. 369 of this Station.

When tubers are left in the sealed jars until their entire surface becomes moist they are usually attacked by anaerobic bacteria which soften the tissue in spots under the skin. In some cases large clusters of gas bubbles appear. The odor of tubers so affected is extremely disagreeable. When exposed to the air they quickly decay. Occasionally, the tubers become moldy in the jars, but, as a rule, bacteria gain the supremacy if the jars are well filled and the temperature at 70° F. or higher. If tubers affected with this bacterial decay are permitted to remain long in a sealed jar pale-straw-colored liquid rapidly collects in the bottom until the jar is about one-half filled with it.

Blackheart may be expected to appear in any tuber which has been long enough in a sealed jar to show moist areas on the surface. Occasionally, it is found also in tubers which show no moisture while in the jar or external discoloration after exposure to the air. In general, the more severe the sweating and external discoloration the greater the severity of the blackheart.

The flesh of tubers cut immediately after removal from a sealed jar is of normal color, but after exposure to the air for a short time the central portion of the cut surface first becomes pink then gradually blackens. Tubers exposed to the air twenty-four hours or longer before cutting show blackheart immediately, if ever.

The tissue affected with blackheart is that which occupies the medullary region. In the main, it lies in the central portion of the tuber, but it is often very irregular in outline. (Plate X.) It is almost always elongated in the direction of the long axis of the tuber and, frequently, branches extend to the buds. (Plate XI.) Occasionally, blackheart appears in the form of large black spots scattered irregularly thru the flesh of the tuber. Sometimes it is accompanied by blackening in the vascular region. The color of affected tissue varies from gray black to coal black.

Tubers may be quite severely affected with blackheart yet sprout normally and produce normal plants. Special attention has been given to the character of the sprouts of tubers affected with blackheart. No tendency to produce spindling sprouts has been observed. If any sprouts appear they are of normal diameter.

When tubers affected with blackheart are exposed for several days to air of low humidity (e. g., that of an ordinary living room) cavities develop in them. Such cavities are often of considerable size and lined with a layer of tough, black tissue resembling rubber. Evidently, these cavities result from shrinkage of the affected tissue thru loss of water as described by Bartholomew. (Plate X.)

Smaller cavities of another kind and of a different origin are found frequently in tubers which have been enclosed in sealed jars for some time but not long enough to produce blackheart. These are usually lens-shaped, three to ten millimeters in diameter, and destitute of a black lining or flesh discoloration of any kind. Altho commonly found near the center of the tuber they may occur near one end or in the vicinity of a bud. Occasionally, two or more cavities are found in a single tuber. They occur much more frequently in small tubers than in large ones. How these cavities originate is not known to the writers, but the fact that they appear only in tubers from which air has been excluded indicates that lack of oxygen is, in some way, responsible for them. Diligent search has been made for such cavities in tubers well supplied with air, but none have ever been found. That they are not caused by shrinkage of the tissue thru loss of water is shown by the fact that they are formed while the tubers are yet in the jar and surrounded by an atmosphere heavily charged with moisture. They are found in tubers cut immediately after removal from sealed jars.

These cavities differ from those known as hollow-heart in being smaller, more regular in shape, free from discoloration of the surrounding tissue, and by occurring in small tubers rather than in large ones. They are of little economic importance.

BLACKHEART PRODUCED AT LOW TEMPERATURE.

The following experiment demonstrates that blackheart may be produced at temperatures below 43° F.:

On December 16, 1915, three glass jars filled with clean, dry, sound tubers were hermetically sealed and placed in the Station potato cellar. One of the jars was opened at the end of twenty-three days. Eight of the twelve tubers which it contained were moist on the surface. Upon exposure to the air they became discolored all over. The other four, which were dry when removed from the jar, did not discolor; but they showed a number of small pits. Eight of the tubers had small areas of blackheart.

A second jar was opened at the end of forty-one days. None of the tubers were moist, but four showed large areas of discoloration while yet in the jar and the others were somewhat pitted. All of the twelve tubers were cut immediately. Eleven of them were found to be severely affected with blackheart. One of these had, also, a cavity. The remaining tuber was normal within.

The third jar was opened at the end of fifty-eight days. All of the tubers were dark-colored externally and wet all over, but none were moldy or affected with bacterial decay. They had an odor of alcohol. All were affected with blackheart and three had, also, good-sized cavities.

The temperature of the air in the cellar during the period covered by this experiment (December 16, 1915, to March 14, 1916) is given in Table IV. The highest temperature was 42° F. and the lowest 34° F.

QUANTITY OF AIR REQUIRED FOR THE MAINTENANCE OF LIFE AND HEALTH IN POTATO TUBERS.

It having been demonstrated that tubers in full, sealed jars soon die and decay the question arose as to how large a volume of air is required for the maintenance of life and health in potato tubers.

It is a well-known fact of plant physiology that potato tubers require a certain amount of air. So long as they live they must respire; that is, they consume oxygen and give off carbon dioxid and water. Their source of oxygen is the air. Hence, they must have a constant supply of air. It is known, also, that, within certain limits, the rapidity of respiration increases with rise in temperature.

At a temperature of 70° F. potato tubers respire more rapidly, consume more oxygen and hence require more air than at a temperature of 50° F. Likewise, more air is required at 50° F. than at 35° F.[4]

Lacking means of controlling the temperature accurately the writers have been unable to secure experimental data which would enable them to give a wholly satisfactory answer to the question here raised. However, it seems worth while to record some of the results obtained in the experiments.

From the results of a few experiments with half-full and quarter-full jars it appears that with these larger quantities of air the tubers behave in practically the same way as tubers in full jars except that the symptoms of blackheart and decay are slower in making their appearance.

AN EXPERIMENT WITH A HALF-FULL TANK AND A HALF-FULL JAR.

In February, 1916, the following experiment was made: A cylindrical, galvanized-iron tank nine inches in diameter and eighty inches deep was half filled with potato tubers (244 tubers weighing 56.5 pounds) and the tank hermetically sealed by means of a tight-fitting cover overrun with melted paraffin. The potatoes were taken from a cellar where they had been in storage at a temperature of 36–42° F. They were washed and thoroly dried before being placed in the tank. Only perfectly sound tubers were used. No sprouts had started on any of them.

At the same time a glass jar (capacity 3.5 quarts) was half filled with some of the same lot of tubers and sealed. This was to be used as an indicator to determine the proper time for opening the tank. A second glass jar of the same kind was completely filled with tubers and sealed. The tank and pair of glass jars were placed in a partially darkened room the temperature of which was recorded once a day. Owing to the temperature being rather low, 13–16° C.,

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[4] Ziegenbein (*Jahrb. Wiss. Bot.* [Pringsheim] **25**:594. 1893.) has determined the rapidity of respiration of potato tubers at different temperatures by measuring the quantity of carbon dioxid produced by a given weight of tubers in a given length of time. His results are stated in milligrams of carbon dioxid produced by 100 grams of tubers in one hour. They are as follows:

Temp. *Deg. C.*	CO_2 *Mg.*	Temp. *Deg. C.*	CO_2 *Mg.*	Temp. *Deg. C.*	CO_2 *Mg.*
10	1.17	35	7.85	50	11.14
20	2.22	40	10.24	55	10.30
30	4.62	45	12.22	60	2.71

the tubers in the full jar did not show the moist areas indicative of distress until the twentieth day which is about twice the time required at a temperature of 21° C.

On February 23, at the end of twenty-one days, the full jar was opened. Of the twenty-one tubers in this jar six were moist over a large part of their surface, ten moist over small areas and the remaining five dry all over. Upon exposure to the air a majority of the tubers became considerably discolored on the surface in the usual manner. Seven hours after removal from the jar all twenty-one of the tubers were cut. In every one the flesh at the center had a pink color which soon changed to black upon exposure to the air. Every one developed a definite case of blackheart.

On March 2, at the end of twenty-nine days, the half-full jar was opened. No sprouts were started. Only four of the eleven tubers showed any moisture on the surface and these only traces; but the appearance of specks of mold here and there on the tubers led to the suspicion that they had begun to deteriorate. The tubers were cut immediately upon removal from the jar. At first, the only indication of an abnormal condition internally was the occurrence, in four tubers, of a small black spot in the flesh near the bud end. Within an hour after cutting, the central portion of all eleven tubers turned pink and within five hours all showed blackheart. On the whole, the blackheart in this lot of tubers was not as pronounced as in the tubers from the full jar, but the difference was not great.

On March 6, at the end of thirty-three days, the half-full tank was opened. The tubers were found to be in bad condition. Nearly all were wet over their whole surface and they had a very disagreeable odor, but there was no free liquid in the bottom of the tank. Altho so wet, the tubers were not softened. There were no rotten spots on them. Some tubers showed a puckering of the skin around the eyes and had a wilted appearance. They were distinctly less turgid than at the beginning of the experiment. Tubers cut immediately upon removal from the tank showed no internal discoloration at first, but within an hour they began to show pink areas which in a few hours more became black. Tubers exposed to the air for two days uncut turned brown over nearly their entire surface and drops of a brownish, viscous liquid were scattered quite plentifully over the discolored areas. Most of the tubers were severely affected with blackheart and completely ruined. They were considerably more injured than the tubers confined twenty-one days in the full jar.

AN EXPERIMENT WITH TWO QUARTER-FULL JARS.

In another experiment two quarter-full jars, containing 6.2 volumes of air to each volume of tubers, were kept sealed thirty-six days at a temperature of 14–18° C. In one jar four of the five tubers bore conspicuous tufts of a whitish mold, but the fifth tuber was free from mold. None of these showed the moist areas which appear on tubers in full jars. After four hours' exposure to the air the four moldy tubers became discolored and pitted on the surface. Many of the pits had a tuft of mold at the center. The fifth tuber appeared normal externally, except that it had not begun to sprout. When cut, all five tubers presented good examples of blackheart, and three of them had, also, large cavities.

Tubers from the second jar were only slightly moldy and did not become discolored externally upon exposure to the air. They were cut immediately upon removal from the jar. Every one had pronounced blackheart with, also, a good-sized cavity. The fact that the tubers were black inside when first taken from the jar appears to confirm the suspicion that the jar was not made perfectly air-tight at the first sealing. A leak being suspected, the jar was sealed a second time about two weeks after the first sealing.

FIRST SERIES OF EXPERIMENTS WITH PARTLY-FULL JARS.

Subsequently, experiments of wider range were made. In one series of experiments thirteen glass museum jars having a capacity of about 3.5 quarts each were used as containers for quantities of potatoes varying in total weight from four to twenty-four ounces. In each jar the ratio of air to tubers was accurately determined.[5] It varied from 4.6 to 33.2 volumes of air per volume of potato tubers. (See Table I.) The tubers used were clean, sound and dry and had not begun to sprout. The experiment was begun on March 10, 1916, at which time the jars were sealed with paraffin and placed in a partially darkened room. The check consisted of twenty tubers in an open jar. During the course of the experiment the temperature fluctuated between 15° and 22° C.

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[5] The ratio of air to tubers, given in the fifth column of Tables I and II, was obtained in the following manner: The tubers were weighed and placed in the jars which were then filled with water, the water being measured (in cubic centimeters) as put in. The volume of the air (= volume of water) thus obtained (Table I, fourth column) was divided by the volume of the tubers which latter was computed by multiplying 26.387 by the weight of the tubers in ounces. (Assuming the specific gravity of Sir Walter Raleigh potatoes to be 1.0744, as given by Stewart and Gloyer in Bulletin 369 of this Station, an ounce of tubers (28.35 grams) occupies 26.387 cc. of space.) Its measurement being completed the water was poured out and the jars and tubers thoroly dried before the commencement of the experiment.

TABLE I.— QUANTITY OF AIR REQUIRED FOR MAINTENANCE OF LIFE IN POTATO TUBERS: FIRST SERIES OF EXPERIMENTS.

JAR.	Number of tubers.	Total wt. of tubers.	Total quantity of air.	Number of volumes of air per volume of tubers.*	CONDITION AT END OF 40 DAYS.	
					Sprouts	Black-heart.
		Oz.	*Cc.*			
I	7	24	2,940	4.6	Barely started; dead	Present.
II	5	20	3,070	5.8	Barely started; dead	Present.
III	4	16	3,105	7.4	Barely started; dead	Present.
IV	3	14	3,190	8.6	Barely started; dead	Present.
V	3	12	3,310	10.5	Very feeble; dead†	Present.
VI	3	11	3,330	11.5	Very feeble; dead	Present.
VII	2	10	3,350	12.7	Very feeble; dead	Present.
VIII	2	9	3,490	14.7	Very feeble; dead	Present.
IX	2	8	3,320	15.7	Feeble; dead	Present.
X	2	7	3,410	18.5	Feeble; dead	Present.
XI	2	6	3,520	22.2	Normal	Present.
XII	2	5	3,550	26.9	Normal	Absent.
XIII	1	4	3,500	33.2	Normal	Absent.

* For the method by which the ratios given in this column were computed, see the footnote on page 331.

† It appears that Jar V was not perfectly air-tight.

The condition of the tubers in the several jars was carefully noted and compared with the check at the end of thirteen, eighteen, twenty-five, thirty-six and forty days.

Sprouts started in all of the jars. With 8.6 volumes or less of air (Jars I–IV) the sprouts were not, at any time, more than barely visible. Some of them remained alive at the end of eighteen days, but all were dead at twenty-five days. In Jars VI–VIII, containing 11.5 to 14.5 volumes of air, sprouts started freely, attained a length of one-fourth inch and lived upwards of twenty-five days; but they were distinctly less robust than the sprouts on check tubers and were either dead or dying at the tips by the end of thirty-six days. In Jars IX–X, containing 15.7 and 18.5 volumes of air, the sprouting of the tubers appeared normal for about eighteen days. At the end of twenty-five days the sprouts were about one-half inch in length when sprouts on the check tubers were from one-half to three-fourths of an inch long. At thirty-six days they were dying at the tips. In Jars XI–XIII, containing 22.2 to 33.2 volumes of air, the sprouts were considerably longer than those on the check tubers, but somewhat less robust, owing, presumably, to the greater humidity of the air in the sealed jars. So far as sprouting is concerned there was no indication that the tubers in these jars suffered from lack of air.

In some of the jars the tubers became damp on the surface, but they did not show distinct moist areas such as appear on tubers in full jars.

In Jars I and IV, containing 4.6 and 8.6 volumes of air, the tubers became quite moldy after twenty-five days of confinement; but in Jars II and III, containing 5.8 and 7.4 volumes of air, there were only traces of mold. The cause of this difference in moldiness is not known. The mold appeared in conspicuous whitish tufts, chiefly at the eyes. It was not identified.

At the end of forty days the jars were all opened and the tubers immediately cut and examined for blackheart. All three tubers in Jar V showed a cavity surrounded by blackheart when first cut. This is considered proof that the jar had leaked air, as had been suspected from the sprouting of the tubers. The tubers in Jar XII (with 26.9 volumes of air) and Jar XIII (with 33.2 volumes of air) showed no indication of blackheart even after exposure to the air. In all other jars the tubers were white inside when first cut, but after exposure to the air for a short time all developed pinkheart, which, in the course of a few hours more, became typical blackheart.

SECOND SERIES OF EXPERIMENTS WITH PARTLY-FULL JARS.

A second series of experiments with partly-full jars included eleven jars of potatoes in which the number of volumes of air per volume of potato tubers varied from 9.2 to 22.1. (See Table II.) This series was started April 21 and continued for twenty-six days, during which time the temperature ranged from 20 to 23.5° C. Very small sprouts were showing when the tubers were placed in the jars.

TABLE II.— QUANTITY OF AIR REQUIRED FOR MAINTENANCE OF LIFE IN POTATOES: SECOND SERIES OF EXPERIMENTS.

JAR.	Number of tubers.	Total wt. of tubers.	Total quantity of air.	Number of volumes of air per volume of tubers.*	Condition of sprouts at end of 26 days.
		Oz.	*Cc.*		
I..........	4	14	3,390	9.2	Barely started; dead.
II.........	3	13	3,200	9.3	Barely started; dead.
III........	3	13	3,250	9.5	Barely started; dead.
IV........	2	10	3,320	12.6	.75 in. long; dead at tips.
V.........	2	10	3,450	13.1	.75 in. long; dead at tips.
VI........	2	7	3,390	18.4	1–1.25 in. long; brown at tips.
VII........	2	7	3,400	18.4	1–1.25 in. long; brown at tips.
VIII.......	2	6.5	3,375	19.7	1.25–1.5 in. long; normal.
IX........	2	6.5	3,425	20.	1.25–1.5 in. long; normal.
X.........	2	6	3,485	22.	1.25–1.5 in. long; normal.
XI........	2	6	3,500	22.1	1.25–1.5 in. long; normal.

* For the method by which the ratios given in this column were computed, see the footnote on page 331.

Tubers having 9.5 volumes or less of air (Jars I–III) barely started sprouts which all died within about twenty days. On tubers having 12.6 and 13.1 volumes of air (Jars IV and V) the sprouts attained a length of over three-fourths of an inch, but they were less robust than the sprouts on check tubers and began to wither at the tips by the end of twenty days. The sprouting of tubers having 18.4 volumes of air (Jars VI and VII) appeared to be normal at twenty days, but at twenty-six days the sprouts had begun to turn brown at the tips. In the remaining four jars (VIII–XI), with 19.7 to 22.1 volumes of air, the sprouting was normal and there was no external evidence of injury up to the end of twenty-six days, when the experiment was closed.

As happened in the first series, the sprouts on tubers having a large volume of air were considerably longer than the sprouts on check tubers, but they were not so stout. For example, at the end of twenty days, in Jars VI–IX, with 18.4 to 22.1 volumes of air, the larger sprouts measured from one to one and one-fourth inches, while the sprouts on check tubers were only three-fourths of an inch long. The greater length of sprouts in the sealed jars is to be attributed to the greater humidity of the air in which they were formed.

No observations were made on the condition of the tubers as regards blackheart.

RELATIVE RESISTANCE OF LARGE AND SMALL TUBERS.

According to Hoffman and Sokolowski [6] small tubers respire more rapidly, volume for volume, than large ones. This being true, it seems as if a scarcity of air should be less injurious to large tubers than to small ones. Two experiments were made in an attempt to demonstrate this experimentally.

In the first experiment four glass museum jars (capacity about 3700 cc.) were completely filled with tubers of different sizes and kept hermetically sealed for a period of sixteen days at a temperature of 57–70° F. In each jar the tubers were fairly uniform in size. Jar I contained 42 tubers having an average weight of 49.3 grams; Jar II, 26 tubers with an average weight of 81.8 grams; Jar III, 26 tubers with an average weight of 100.3 grams; and Jar IV, six tubers with an average weight of 293 grams. By putting small stones into some of the jars the ratio of air to tubers was made uniform in all four jars, viz., 22.5 cc. of air to each ounce of tubers, or .85 of a volume of air per volume of tubers.

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[6] Abstract in *Exp. Sta. Rec.* 24:541. 1911.

When the jars were opened at the end of sixteen days the condition of the tubers in Jars I–III appeared quite similar. All were quite damp and foul-smelling, tho of normal color and sound, with the exception of two tubers, each of which had a small bacterial soft-rot spot. No sprouts had started. Sixteen hours after removal all tubers were showing large areas of discoloration occupying one-fourth to three-fourths of their entire surface, the tubers in all three jars being about equally affected on the average. Subsequent observations showed that none of the tubers were capable of sprouting and about one-half of the total number were affected with blackheart.

The large tubers in Jar IV were affected quite differently. They were dry when the jar was opened, did not smell bad, and upon exposure to the air they did not discolor over large areas, but, instead, became covered with small, sunken, dead, brown spots similar to those which result from fumigation with formaldehyde gas.[7] Altho most of the spots were less than one-fourth inch in diameter and few of the eyes were affected, the spots were so numerous that the tubers were severely injured. Nevertheless, five of them sprouted fairly well, producing short, thick sprouts. After eighteen days' exposure to the air five of the tubers were cut. One was normal within, but in each of the other four there was a large cavity lined with a layer of dead, black tissue — typical blackheart in the late stage.

In the second experiment six jars were used. Jars A and B were filled with small tubers having an average weight of 69 grams in A and 84 grams in B; Jars C and D with large tubers having an average weight of 288 grams in C and 349 grams in D; Jar E with one large tuber weighing 340 grams and nineteen small ones having an average weight of 78 grams; and Jar F with one large tuber weighing 326 grams and eighteen small ones having an average weight of 83 grams. The ratio of air to tubers was made uniform in all six jars, viz., 28.62 cc. of air to each ounce of tubers or 1.1 volumes of air per volume of tubers.

The temperature being somewhat higher, the tubers began to show signs of injury earlier than in the preceding experiment. The jars were opened at the end of twelve days. While the appearance of the tubers in the several jars varied considerably the differences could not be ascribed to differences in the size of the tubers. The large tubers in Jar D were as severely injured as the small tubers in Jars A and B and in the same manner. They were damp and free from mold when first removed from the jars, and showed large areas of discoloration upon exposure to the air. On the other hand, the large tubers in Jar C were dry and somewhat moldy when taken from the jar, and upon exposure to the air developed sunken, brown spots as if injured by formaldehyde gas. The large tuber in Jar E was quite as severely injured as the small tubers in the same jar and in the same manner. All had large areas of surface discoloration. On the other hand, the tubers in Jar F, both large and small ones, showed considerably less surface discoloration and more spotting of the gas-injury type. Some of the small tubers in Jar F were no more injured than the large tuber in that jar.

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[7] See Bulletin 369 of this Station.

None of the tubers in any of the jars were able to sprout. All of them were more or less affected with blackheart, but there were no differences in this respect worthy of mention.

While the first of these experiments indicates that large tubers are more resistant to asphyxia than are small ones, the second experiment leaves this in doubt and suggests that some other character or condition of the tubers is more important than size.

The view that the size of a tuber is less important than some other character in determining its susceptibility to injury from an insufficient supply of air is supported by the observation that when tubers of mixed sizes are confined in sealed jars some tubers are affected much earlier and injured more severely than others, and the size of the tubers appears to bear no relation to the earliness or severity of the injury. This has been observed in many of the experiments. With respect both to blackheart and surface discoloration there is marked individuality among tubers, but the explanation of it is not known.

DEEP PILING OF POTATOES IN STORAGE: EXPERIMENTS WITH POTATOES IN DEEP CYLINDRICAL TANKS.

In storage cellars potatoes are often piled to a depth of several feet without any provision being made for ventilation. Having learned from the experiments with sealed jars that potatoes require a considerable amount of air it became of interest to the writers to know how deeply potatoes may be piled without danger of injury from asphyxiation. It seems as if the small amount of air available to tubers at the bottoms of deep piles might be insufficient for the requirements of respiration, particularly if the temperature is high.

Information on this point was sought from experiments in which potatoes were piled to depths of 6.5 to 10.5 feet in cylindrical, galvanized-iron tanks which were air-tight on the bottom and sides but wide open at the top. The diameter of these tanks was nine inches in all cases, but the hight varied from 80 to 126 inches. How the aeration of tubers in these tanks compares with that of tubers at an equal depth in large piles and bins is not fully known. Certainly, the aeration was no better in the tanks than in piles; because in the tanks air could reach the tubers only from the top while in piles there must be some circulation of air on the sides as well as from the surface. However, when it is considered that the air which tubers in piles receive from the side has been robbed of most or all of its oxygen by neighboring tubers it appears improbable that such air is of much value. Accordingly, there is reason to believe that the behavior of tubers in the tanks represents fairly well the behavior of tubers in piles at a similar depth.

FIRST TANK EXPERIMENT.

In the first of these tank experiments a single tank eighty inches deep was used. It was filled and the experiment begun on February 16, 1915. As the potatoes were being put into the tank pieces of wire screen were inserted at depths of 54 and 27 inches to divide the contents of the tank into three parts. The tank was placed in an unheated interior room of an office building where the humidity of the air was low and the temperature 18–21° C. most of the time.

On April 27, at the end of seventy days, the potatoes were taken out of the tank. Altho the tank was more than even full at the beginning of the experiment the uppermost tubers were now seven or eight inches below the top of the tank. Tubers on top were somewhat shriveled. They had sprouts one to two inches long. (Plate XIII, fig. 1.) At a depth of two feet the tubers were sprouting vigorously. Some sprouts were short and much branched; others three to four inches long and club-shaped. (Plate V, fig. 2.) All of the sprouts, excepting those on tubers near the top of the tank, were covered with a conspicuous eruption of enlarged lenticels.

Down to the first division screen (depth of 27 inches) the tubers were dry, free from mold and decay, sprouting normally and the sprouts all alive. When cut, none of the tubers were found affected with blackheart or other internal discoloration. Two had hollowheart, but no cavities were found like those described on page 283.

As soon as the first screen was passed the tubers began to be damp and moldy and the sprouts to show signs of decay. The prevalence of moisture, mold and decay increased rapidly until by the time the second division screen was reached (at the depth of 54 inches) many tubers were ruined. (Plate XIV, fig. 2.) When the tubers were cut no case of blackheart was found. A few tubers showed some blackening in the vascular region and one had a lens-shaped cavity.

Below the second screen, at a depth of 54 to 80 inches, the tubers were all ruined. A few at the very bottom had barely started sprouts. (Plate XIV, fig. 1), but most of the tubers had produced robust sprouts which afterward decayed. There were no indications of spindling sprouts. All of the tubers were very wet and there was about a pint of foul-smelling liquid in the bottom of the tank. Upon exposure to the air the tubers became discolored on the surface like tubers from sealed jars. When cut, about fifty per ct. of the tubers showed pronounced blackening in the vascular region, a considerable number had small cavities like those described on page 283 and some were affected with blackheart. Both blackheart and cavity formation were usually accompanied by fibro-vascular blackening.

While this experiment demonstrates insufficient aeration of the tubers in the lower part of the tank the effect on the tubers was not quite what was expected. There was less blackheart and much more fibro-vascular blackening. Neither before nor since have we observed so much fibro-vascular blackening to result from insufficient aeration.

SECOND TANK EXPERIMENT.

A second tank experiment was started on April 29, 1915, using the same tank as in the first experiment after disinfection with formaldehyde solution. It being desired to ascertain the temperature of the tubers within the tank a glass tube one-half inch in diameter and eighty inches long was placed in the center of the tank and the tubers filled in around it. When the tank was completely filled the glass tube was raised a few inches and a thermometer attached to a string was lowered thru the tube until its bulb rested among the tubers five inches from the bottom of the tank. The upper end of the tube was kept closed with a rubber stopper except for short intervals twice a day when the thermometer was drawn up for reading. A second thermometer, previously compared with the first, was hung in the air on the outside of the tank five inches above the floor.

In the fifteen days during which the experiment was conducted the thermometers were read 25 times. In fourteen readings the thermometer inside the tank stood higher than the one on the outside, but in no case was the difference greater than 1° C. In the other eleven readings the two thermometers agreed. The highest temperature recorded was 25° C.; the lowest, 19° C.

From this it appears that the temperature inside the tanks in these experiments is but slightly higher than that of the surrounding air. The injury to the tubers is not due to overheating.

The potatoes were removed from the tank on April 14 at the end of fifteen days. Tubers near the top had sprouts about three-fourths of an inch long. The maximum vigor of sprouting had occurred, as in the first experiment, at a depth of 24–27 inches. Here, most of the sprouts were an inch long. (Plate XV, fig. 1.) Tubers in the bottom of the tank were only slightly damp, free from mold and apparently sound; but the sprouts were barely started and in the same condition as at the beginning of the experiment. (Plate XV, fig. 2.) It was plain that sprouting had been checked by lack of air. Altho apparently sound when removed from the tank, these tubers within a few days developed rotten spots of quite large size and extending deeply into the flesh. Some which were given an opportunity to sprout produced normal sprouts. None of the tubers were affected with blackheart.

Tubers in a full sealed jar, but in other respects under conditions parallel with those in the tank, were dead at the end of seven days. Upon exposure to the air they discolored badly, became affected with blackheart and were unable to sprout.

THIRD TANK EXPERIMENT.

A third experiment with the 80-inch tank was begun October 4, 1915, and closed October 28. The potatoes used in this experiment had been out of the ground only two weeks and so had not completed their rest period. Accordingly, it was not expected that they would sprout; and, as a matter of fact, they showed no sign of sprouting at the end of 24 days notwithstanding the temperature was sufficiently high (15–19.5° C.) to induce sprouting had the tubers been capable of it. Tubers in the bottom of the tank were slightly moist, but showed no discoloration (either internal or external) or any other indication of injury.

FOURTH TANK EXPERIMENT.

In the fourth experiment the potatoes were put into the tanks November 8, 1915, and taken out January 6, 1916, at the end of 59 days. Three tanks were used — two 80 inches deep and one 107 inches deep. The experiment was conducted in the same room as the previous experiments.[8] The ceiling, being only 109 inches from the floor, was barely high enough to admit the tall tank.

The tall tank was divided into four sections by wire screens placed at depths of 81, 54, and 27 inches, the object being to make it possible to keep the tubers from different parts of the tank separate while removing them from the tank. Similarly, the shorter tanks were divided into three sections each. In both of the 80-inch tanks glass tubes were installed for the insertion of thermometers as in the second experiment. One of the 80-inch tanks was set in a wooden box 18 inches square by 82 inches deep and the space between the tank and walls of the box filled with sawdust to secure insulation.

Four thermometers were used — one inside the insulated tank with its bulb four inches from the bottom; one in the same position on the inside of the uninsulated 80-inch tank; another outside the tanks four inches above the floor; and the fourth outside the tanks ten inches below the ceiling. Parallel readings of the four thermometers were made daily. Twenty-three of these readings were made at 8 A. M., eighteen at 1 P. M. and seventeen at 5 P. M.

Table III gives the maximum and minimum temperatures recorded by each of the four thermometers at 8 A. M., 1 P. M. and 5 P. M.; also, the average temperature at each of these hours. From an examination of this table it will be seen that the average temperature inside the two tanks differed but slightly from that of the air of the room at the same distance above the floor. This confirms the conclusion deduced from the second tank experiment, viz., that the injury to tubers in the tanks is not due to overheating. That heat is evolved in the process of respiration is a well-established fact; but the amount of heat resulting from the respiration of potatoes is, seemingly, too small to materially affect their keeping qualities. This is shown by the fact that the average temperature within the insulated tank was practically the same as that of the surrounding air. However, it must be admitted that differences in the condition of the tubers in different tanks in this experiment, about to be described, indicate that a slight rise in temperature continued for a long time may have important effects.

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[8] The room in which these experiments were conducted is one formerly used for experiments on the curing of cheese. It was built expressly for that purpose and constructed in such a way as to facilitate the maintenance of a constant temperature. At first it was equipped with an automatic device for regulating the temperature, but at the time of our experiments this was not in operation. The room is an unheated interior one, 10x10x9 feet, with a single double-glass window in the upper part of the door. The walls, floor and ceiling are thoroly insulated. The door, also, is insulated and fits very closely.

TABLE III.— SUMMARY OF TEMPERATURE CONDITIONS AFFECTING POTATOES IN TANKS: FOURTH EXPERIMENT.

LOCATION OF THERMOMETER.	MAXIMUM AND MINIMUM TEMPERATURES.						AVERAGE TEMPERATURE.		
	8 A. M.		1 P. M.		5 P. M.		8 A. M.	1 P. M.	5 P. M.
	Max.	Min.	Max.	Min.	Max.	Min.			
	Deg. C.	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*	*Deg. C.*
Inside insulated tank near bottom.....	19	15.5	19	15.5	18.5	15	16.6	16.6	16.7
Inside uninsulated tank near bottom.	19.5	14	19	14.5	19	14	16.2	16.3	16.5
Outside the tanks near floor........	20	14	20	14	20	14	16.2	16.6	16.9
Outside the tanks near ceiling......	20	14.5	20.5	14	20.5	15	16.6	17.7	17.6

At the end of 59 days the potatoes were removed from the tanks. In describing the results of the experiment the portion of each tank above the upper division screen will be designated as Section 1; that between the upper screen and the next one below, as Section 2; and so on to the bottom of the tank.

The two outstanding features of the experiment were: (1) The sprouts on the tubers in Section 1 of the uninsulated tank were noticeably shorter than the sprouts on tubers in the corresponding section of the other two tanks; (2) tubers in Section 3 of the tall tank were sprouted less and were considerably more affected with rot than tubers in the corresponding section of the two 80-inch tanks.

A third feature, less conspicuous yet worthy of note, was the better condition of the tubers in Section 3 of the uninsulated tank as compared with that of tubers in Section 3 of the insulated tank.

In Section 1 of the uninsulated tank the longer sprouts were one-half to two-thirds of an inch long, while those in Section 1 of the other two tanks were about twice this length. (Plate XVI.)

In Section 3 of the tall tank no sprouts had started. Many tubers had sunken rotten spots or areas of brown discoloration accompanied by softening of the tissue. In Section 3 of both short tanks the tubers had begun to sprout some and several had rotten spots; but on the whole the condition of the tubers was considerably better than that of tubers in Section 3 of the tall tank.

It is hard to believe that the slightly higher temperature in the insulated tank can have been responsible for the greater length of the sprouts in Section 1 as compared with those in the corresponding section of the uninsulated tank; but there seems to be no other satisfactory explanation for it. Likewise, there appears to be no other explanation for the better condition of the tubers in the uninsulated tank.

The greater length of sprouts in the upper section of the tall tank may be attributed to the fact that the tubers were nearer the ceiling where the temperature was slightly higher.

As regards the better condition of the tubers in Section 3 of the short tanks as compared with those in Section 3 of the tall tank the only plausible explanation appears to be that the daily raising and lowering of the thermometers thru the glass tubes in the short tanks permitted the access of a small amount of air giving the tubers in these tanks slightly better aeration.

The tubers in Section 4 of the tall tank (at a depth of 81–107 inches) were in bad condition. Many were affected with a brown soft rot, some were moldy and others were discolored. Upon exposure to the air at a temperature of 70° F. for two weeks over two-thirds of the tubers either decayed or developed large rotten areas; but none were affected with blackheart, and those which did not decay produced normal sprouts.

FIFTH TANK EXPERIMENT.

A fifth tank experiment was begun January 15, 1916. It consisted of an 80-inch tank (A) insulated by packing sawdust around it as in the preceding experiment, an uninsulated 80-inch tank (B), an uninsulated 107-inch tank (C), an 80-inch tank (D) hermetically sealed, and three large glass museum jars hermetically sealed. All were filled with clean, dry, sound potatoes. By means of wire screens each of the 80-inch tanks was divided into three sections and the tall tank into four sections as in the fourth experiment. The experiment was conducted in the same room as previous ones. The temperature of the room was taken, near the floor and near the ceiling, at least once a day and sometimes oftener. Thirty-five readings, made about 8 A. M., showed an average temperature of 14.5° C. at the floor and 15.4° C. at the ceiling. Twenty-three readings, made about 1 P. M., averaged 15.3° C. at the floor and 16.2° C. at the ceiling. Fifteen readings, made about 5 P. M., averaged 15° C. at the floor and 16.3° C. at the ceiling. The minimum floor temperature was 11° C.; the maximum ceiling temperature 22° C. Accordingly, the temperature averaged a little lower in this experiment than in the preceding one.

The tubers in the sealed jars were used as indicators to determine the proper time to open the sealed tank D. The ratio of air to tubers in these jars was not determined; but judging from previous determinations it must have been slightly over one volume of air per volume of tubers. In two of the jars the tubers behaved in the usual manner. One was opened at the end of fourteen days, the other at sixteen days. The tubers were quite damp when first removed. A few hours' exposure to the air caused them to discolor badly on the surface and, when cut, they were found affected with blackheart. The tubers in the third jar behaved somewhat differently.[9] They were much freer from moisture. When removed at the end of seventeen days only twelve of them were slightly damp; the other ten were dry. None of the twenty-two discolored externally upon exposure to the air. Nevertheless, all of the tubers but one developed pronounced cases of blackheart. Altho wholly destitute of sprouts at the time of their removal from the jar, the tubers afterward developed normally robust sprouts.

Tank D was provided with a cover which fitted closely into the upper end with its upper surface one-fourth inch below the level of the rim of the tank. A narrow flange on the inside of the tank prevented the cover from slipping down. Melted paraffin was run over the cover to a depth of one-fourth inch to make it air-tight. The tank contained 550 tubers weighing 113 pounds. The volume of the air among the potatoes was measured and found to be 37.5 liters, which is 20.74 cc. per ounce of tubers, or .78 of a volume of air per volume of tubers.

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[9] It is possible that this jar was not perfectly air-tight.

Tank D was opened at the end of sixteen days. The condition of the tubers was similar to that of tubers in the glass jar opened at the same time. An interesting feature was the greater injury to tubers in the bottom of the tank. Upon taking the tubers from the tank it was noticed that the dampness of the tubers increased from the top of the tank downward. A large majority of the tubers in the upper section were moist over only a part of their surface. A few were moist all over and a few were dry all over. In Section 3 many tubers were moist all over. A considerable number had dry spots, but it is doubtful if there were any which were dry all over. However, there was no free liquid in the bottom of the tank. Neither were any of the tubers moldy or rotten. Upon exposure to the air the tubers from Section 3 showed considerably more surface discoloration than did the tubers from Section 1. In Section 3 tubers wholly free from surface discoloration were rare, while in Section 1 such tubers were frequent. Tubers from Section 2 were in an intermediate condition as regards both dampness and surface discoloration. Blackheart was general and in a majority of the tubers it was severe. (Plate XI.) Only eight of the 550 tubers were entirely free from blackheart. The severity of the blackheart was but slightly greater in Section 3 than in Section 1.

If the cover of Tank D were not air-tight the lesser degree of injury in Section 1 would be accounted for; but there is nothing to indicate that the cover did not fit perfectly air-tight. The tubers were affected in the same manner and as severely as those in an air-tight jar under parallel conditions. Presumably, the carbon dioxid gas produced in respiration accumulated in the bottom of the tank. This may have been responsible. Perhaps, the accumulation of CO_2 brought about earlier and more complete oxygen starvation in this part of the tank. The only other respect in which Section 3 differed from Section 1 was in the matter of temperature. Section 3, being nearer the floor, was slightly cooler; but the effect of lower temperature would be to lessen the injury, not to increase it.

The other three tanks, A, B and C, were opened at the end of sixty-two days. The tubers in Sections 1, 2 and 3 of the insulated Tank A were in almost exactly the same condition as the tubers in corresponding sections of the uninsulated tall Tank C, which will be described presently. Apparently, the insulation of Tank A had no influence.

Finding the tubers in the bottom of Tank B (uninsulated) sprouting as vigorously as those in the upper section led us to suspect that this tank leaked near the bottom. By filling the tank with water this suspicion was confirmed. Hence, the results of Tank B are of no value except to prove that it is lack of air rather than lower temperature which prevented the sprouting of tubers in the lower portion of Tanks A and C.

The condition of the tubers in the tall Tank C was as follows: Thruout Section 1 the sprouts were 1.5–5 inches long. Many were 3–5 inches long. In the upper half of Section 2 the sprouts were as long as those in Section 1, but in the lower half they were considerably shorter. In the upper half of Section 3 scarcely any sprouts were more than one-half inch long; while in the lower part of this section the sprouts had barely started. In Section 4, also, the sprouts were barely started.

The great differences in the sprouting of tubers at different depths in the tall tank are strikingly shown in Plates XVII and XVIII. Plate XVII, fig. 1, shows the length and the character of the sprouts near the surface of the upper section. The sprouts are thickly covered with short hairs. The sprouts shown in Plate XVII, fig. 2, are from tubers in the upper part of Section 2, at a depth of about 27 inches. Plate XVIII, fig. 1, shows the character of the sprouts at a depth of 54 inches, and Plate XVIII, fig. 2, shows the sproutless condition of tubers at a depth of 80–107 inches. Notwithstanding that the temperature was lower in this experiment than in the preceding one, the tubers produced much longer sprouts in practically the same length of time, owing, probably, to the longer period of rest which the tubers had had at the time the experiment was begun.

In all four sections some of the tubers were slightly moist when first taken out, but none were moldy or rotten. At the time, it was thought that the tubers in the bottom section had suffered no injury, but after a few days' exposure to the air some of them developed sunken, rotten spots which appeared to be a result of their confinement. However, none were affected with blackheart. It is worthy of note that one-half bushel of tubers from the bottom section which were set aside to sprout produced normally robust sprouts. So long as they were confined in the tank at a depth of 80–107 inches they were unable to start sprouts, but when removed from the tank and supplied with air they proceeded to sprout in a perfectly normal manner.

A comparison of the condition of the tubers in the sealed Tank D with that of tubers in the bottom of the tall Tank C leads to the conclusion that there must be considerable circulation of air among potatoes at a depth of seven to nine feet. Tubers in the sealed tank were ruined by suffocation in sixteen days, while tubers in the bottom of the unsealed tank were alive and in fairly good condition at the end of sixty-two days. Meanwhile, the temperature was comparatively high, so that the tubers must have respired quite rapidly.

SIXTH TANK EXPERIMENT.

In the sixth tank experiment only two containers were used — one 107 inches and the other 126 inches deep (or tall). The 107-inch tank was the " tall " tank used in the fourth and fifth experiments. The 126-inch tank was a new one. On account of the height of the taller tank it was necessary to conduct this experiment in a basement room having a high ceiling. The tanks were filled and the experiment begun March 24, 1916. As in previous experiments, pieces of wire screen were used to divide the contents of the tanks into sections about 27 inches long.[10] The tubers used were clean, dry and sound and had not begun to sprout. The temperature was taken at two points — 18 inches below the top of the taller tank and six inches above its base. Thirty-eight daily readings averaged 16° C. for the lower point and 21.6° C. for the upper one. The maximum temperature of 25° C. was registered on the last day of the experiment.

The tubers were removed from the tanks on May 9, at the end of forty-six days. The condition of tubers in corresponding sections of the two tanks varied somewhat owing, probably, to differences in temperature; but the variation is so small that it is unnecessary to give the details for both tanks. It will be sufficient to describe the contents of the 126-inch tank.

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[10] The upper section in the taller tank was only 18 inches long.

Thruout Section 1 the sprouts were about an inch long. There was no mold or indication of decay. Tubers near the top were somewhat shriveled. None of the tubers had blackheart or internal cavities.

In the upper part of Section 2, at a depth of about 2 feet, the longest sprouts in the tank were found. They measured 1–3 inches. Many of them had begun to decay. In the lower part of Section 2, at a depth of 45 inches, the sprouts were only one-fourth to one-half inch long. The tubers were slightly moist. Of twenty-five tubers which were cut immediately, all except two were normal within. These two, after exposure to the air, showed a little blackheart. After twenty-four hours' exposure to the air a few of the remaining tubers showed rotten spots, but the great majority appeared normal. Upon cutting, two were found to contain cavities and three had blackheart.

In Section 3 some of the tubers had started no sprouts; others had produced short sprouts which had afterward decayed. The tubers in this section were characterized by their moldiness. Almost every tuber was covered with white mold which had attacked the eyes, causing a kind of eye-rot. The tubers were a little moist but not wet. There was no disagreeable odor. Of twenty-five, cut immediately, seven had large cavities narrowly lined with black tissue. After exposure to the air these developed blackheart. Eleven of the twenty-five appeared normal when first cut, but later they developed blackheart. The other seven had no cavities and showed little or no blackheart, even after exposure to the air. Most of these tubers came from a depth of about 72 inches.

After exposure to the air for twenty-four hours only a few of the remaining tubers were free from discoloration and blemishes. Several had large rotten spots and nearly all had sunken areas of dead tissue around the eyes. Sixty-six out of seventy-five had blackheart, and many had, also, cavities. The other nine had neither blackheart nor cavities.

In Section 4, at a depth of 72–99 inches, none of the tubers had sprouted. They were wet, but free from mold. The great majority were solid. A few were affected with a soft, foul-smelling bacterial decay. Of twenty-five, cut immedately, twenty had large cavities, most of which had a black lining, and a few of the tubers had traces of blackheart in addition. After a few hours' exposure all twenty had good cases of typical blackheart. The other five tubers lacked cavities, but upon exposure to the air they developed blackheart.

The remaining tubers from Section 4 were exposed to the air for twenty-four hours. By this time a considerable number were partly rotten. On many of them large areas of the surface were badly discolored (nearly black) and frequently slightly sunken. There was, also, considerable pitting and spotting resembling the injury produced by formaldehyde gas. Seventy-five additional tubers were cut. All had blackheart and many had, also, large cavities.

In Section 5 the tubers were very wet and there was a considerable quantity of a foul-smelling liquid in the bottom of the tank. The great majority of the tubers were still solid. A few were affected with bacterial decay. There was no mold. Twenty out of twenty-five tubers cut immediately had large black-lined cavities. Upon exposure to the air these tubers all developed blackheart. Two of the remaining five had each a large area of typical blackheart (also a cavity) when first cut. The other three were free from cavities and blackheart when first cut, but developed blackheart upon exposure to the air.

After twenty-four hours' exposure to the air the external appearance of the remaining tubers of this lot was decidedly superior to that of the tubers from Section 4. They were less discolored and less affected with rot. A considerable number were free from external blemishes. However, all had pronounced blackheart and many contained, also, cavities.

Tubers in this experiment were much more severely injured at the end of forty-six days than were tubers in the preceding experiment at the end of sixty-two days. This must be attributed, chiefly, to the higher temperature in the present experiment. Probably, the higher rate of respiration due to the greater age of the tubers was a minor factor.

The blackheart found among tubers in the open tanks in this experiment was like that produced in sealed jars, except that it was often accompanied by cavities which were already black-lined when the tubers were first taken out. The progressive change in color, from white thru pink to black, was exactly like that which takes place in tubers which have been asphyxiated in sealed jars.

SEVENTH TANK EXPERIMENT.

The seventh tank experiment was a particularly instructive one for two reasons, viz., (1) It was conducted in a regular potato cellar under conditions like those in which potatoes are often stored; and (2) It furnished a basis for the interpretation of the unexpected results obtained in the outdoor pit experiment described on page 356.

TABLE IV.— TEMPERATURE RECORDS: SEVENTH TANK EXPERIMENT AND OUT-DOOR PIT EXPERIMENT.

TANK EXPERIMENT IN CELLAR.			OUT-DOOR PIT EXPERIMENT.				
Date.	Temperature.		Date.	Temperature of outside air.		Temperature inside pit.	
	Deg. F.	*Deg. C.*		*Deg. F.*	*Deg. C.*	*Deg. F.*	*Deg. C.*
Nov. 18....	42	5.5	Nov. 18....	37.5	3.1	39.5	4.2
Nov. 22....	43	6.1	Nov. 27....	53	11.7	42	5.5
Nov. 27....	45	7.2	Dec. 2....	35	1.7	42	5.5
Dec. 1.....	43	6.1	Dec. 11....	26	—3.3	42	5.5
Dec. 16.....	36	2.2	Dec. 18....	34	1.1	42	5.5
Jan. 3.....	41.5	5.3	Jan. 3.....	31	—0.5	42	5.5
Jan. 5.....	41	5	Jan. 12.....	33	0.5	41	5
Jan. 7.....	40.5	4.7	Jan. 19.....	22	—5.5	38	3.3
Jan. 12.....	40	5	Jan. 22.....	51	10.5	38	3.3
Jan. 18.....	37	2.8	Jan. 28.....	42	5.5	39	3.9
Jan. 22.....	41	5	Jan. 31.....	58	14.4	40	4.4
Jan. 26.....	42	5.5	Feb. 14....	9.5	—12.5	39.5	4.2
Feb. 14.....	34	1	Feb. 21....	10	—12.2	37	2.8
Feb. 17.....	35	1.7	Feb. 28....	17	—8.3	37	2.8
March 2...	34	1.1	March 7....	46	7.8	36.5	2.5
March 7...	36.5	2.5	March 11...	25	—3.5	36.5	2.5
March 10...	35	1.7	March 27...	39	3.9	36.5	2.5
March 25...	36	2.2	April 3....	41	5	37.5	3.1
March 27...	36	2.2	April 7....	39	3.9	38	3.3
April 25....	44	6.7	April 13....	42	5.5	39	3.9
May 9.....	48	8.8	April 24....	55	12.8	44	6.7
May 11.....	48.5	9.2	May 1....	60	15.5	47	8.3
May 19.....	49	9.4	May 5....	63	17.2	51	10.5
May 26.....	52	11.1	May 8....	72	22.2	53	10.5
....	......	......	May 15....	......	......	56.5	12.5
....	......	......	May 20....	64	17.8	56.5	12.5
....	......	......	May 25....	72	22.2	56.5	12.5
....	......	......	May 26....	71	21.7	57	12.8

Three 80-inch tanks (D, E and F) were put into the Station potato cellar and filled with potatoes on November 8, 1915. Each tank was separated into three sections in the usual manner. A Fahren-

EXPLANATION OF PLATES.

ALL FIGURES NATURAL SIZE.

PLATE IX.— POTATO TUBERS WITH BROWN SURFACE DISCOLORATION.

Tubers enclosed in a full sealed jar for nine days at a temperature of 68–75° F. Photographed after four hours' exposure to the air. The entire bud end of the tubers is covered by a shallow, brown discoloration.

PLATE X.— BLACKHEART OF POTATOES CAUSED BY EXCLUSION OF THE AIR.

Tubers enclosed in a full sealed jar for ten days at a temperature of about 70° F. and afterward exposed to the air for seven days at the same temperature. Figs. 1–3 are cross sections; Fig. 4, a longitudinal section. In each there is a well-marked cavity at the center which shows best in Fig. 1. All were sprouting.

PLATE XI.— BLACKHEART OF POTATOES CAUSED BY EXCLUSION OF THE AIR.

Longitudinal sections of four tubers which were enclosed in a full sealed tank (Tank D of the fifth tank experiment) sixteen days at a temperature of 53–68° F. and afterward exposed to the air 27 hours. The width of the dark-colored zone on the margins of the sections at the bud end (upper end) shows the depth of the surface discoloration.

PLATE XII.— BLACKHEART OF POTATOES CAUSED BY EXPOSURE TO HIGH TEMPERATURE.

Cross sections of six tubers exposed in open jars within an incubator for 18–40 hours to a temperature of 108–112° F. and then removed to a temperature of about 70° F. The tubers shown in Figs. 1 and 2 were 18 hours in the incubator and 24 hours out; those in Figs. 3 and 4, 24 hours in the incubator and 18 hours out; and those in Figs. 5 and 6, 40 hours in the incubator and 2 hours out. Short sprouts on the tubers at the beginning of the treatment were dead on all. Otherwise, the tubers shown in Figs. 1–4 were normal externally; but those shown in Figs. 5 and 6 had a few small areas of surface discoloration and numerous small, sunken, brown spots resembling injury by formaldehyde gas. In the tubers shown in Figs. 5 and 6 the central portion was gray black instead of brownish black as in the others.

PLATE XIII.— CONDITION OF TUBERS IN FIRST TANK EXPERIMENT.

These tubers were exposed to a temperature of 64–70° F. for 70 days.

Fig 1. Average condition of the tubers near the top of the tank.

Fig. 2. The large club-shaped sprouts common at a depth of two feet. They are prominently roughened with enlarged lenticels. Compare Plate XIV.

PLATE XIV.— CONDITION OF TUBERS IN FIRST TANK EXPERIMENT.

Fig. 1. A tuber showing the condition at a depth of 80 inches. Sprouts barely started, then died.

Fig. 2. A moldy and decaying tuber showing the condition at a depth of 53 inches. Compare Plate XIII.

PLATE XV.— IMPERFECT AERATION CHECKS THE SPROUTING OF POTATOES.

Two tubers from the second tank experiment in which they were subjected to a temperature of 66–77° F. for 15 days.

Fig. 1. A tuber from a depth of two feet.

Fig. 2. A tuber from a depth of 80 inches. Subsequently, upon exposure to the air, this tuber sprouted normally.

PLATE XVI.— SPROUTS FROM POTATOES IN FOURTH TANK EXPERIMENT.

A. Sprouts from Section 1 of uninsulated tank.
B. Sprouts from Section 1 of insulated tank.
C. Sprouts from Section 1 of tall tank.

PLATE XVII.— SPROUTING OF POTATOES IN UPPER PART OF TANK C IN THE FIFTH TANK EXPERIMENT.

Fig. 1. Representative sprouts from near the top of the tank. They are thickly covered with short hairs.

Fig. 2. Representative sprouts from a depth of 27 inches. Destitute of hairs, but rough with enlarged lenticels. Compare Plate XVIII.

PLATE XVIII.— SPROUTING OF POTATOES IN LOWER PART OF TANK C IN THE FIFTH TANK EXPERIMENT.

Fig. 1. A tuber showing the character of the sprouts at a depth of 54 inches.

Fig. 2. An unsprouted tuber from Section 4 showing the sproutless condition of tubers at a depth of 80–107 inches. This tuber and many others like it in Section 4 sprouted normally, later, when exposed to the air. Compare Plate XVII.

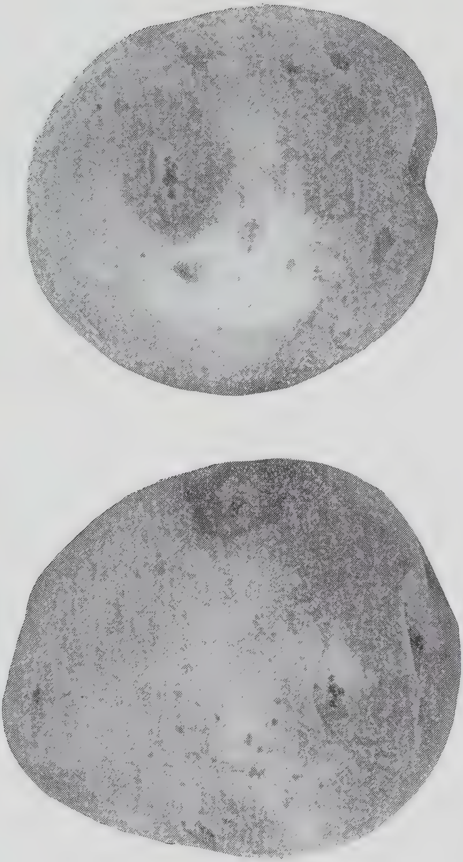


Plate IX.— Potato Tubers with Brown Surface Discolorations.

(For explanation of plate, see page 307.)

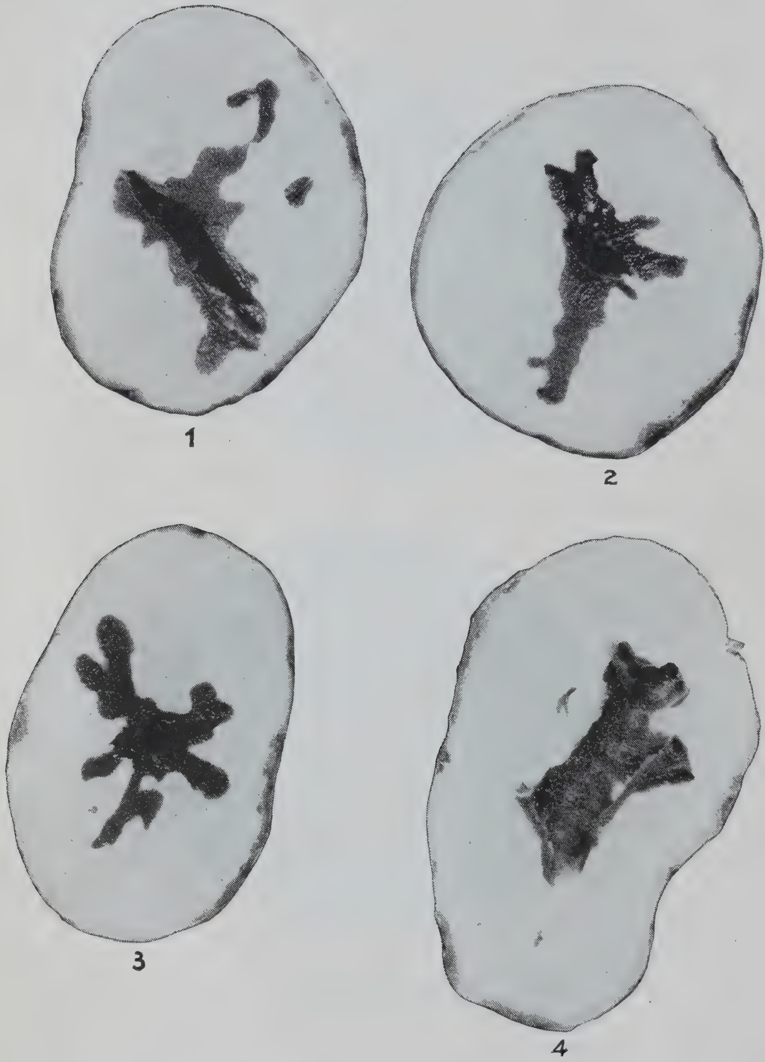


Plate X.— Blackheart of Potatoes Caused by Exclusion of the Air.
(For explanation of plate, see page 307.)

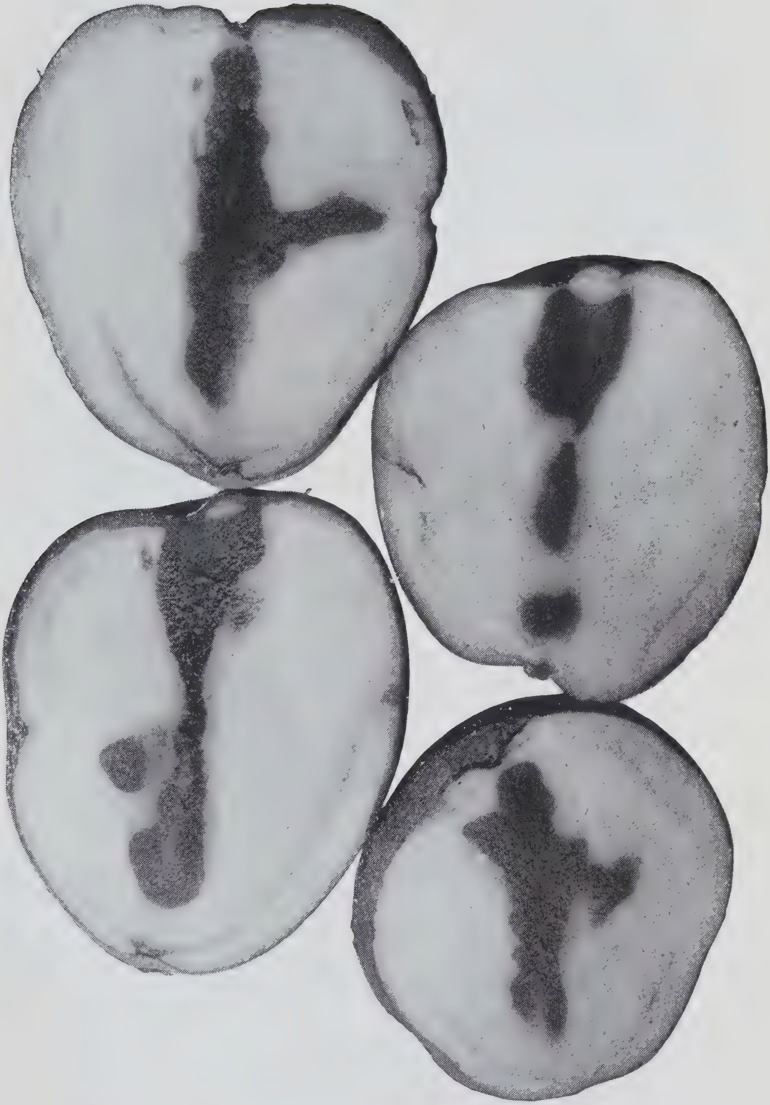


Plate XI.— Blackheart of Potatoes Caused by Exclusion of the Air.
(For explanation of plate, see page 307.)

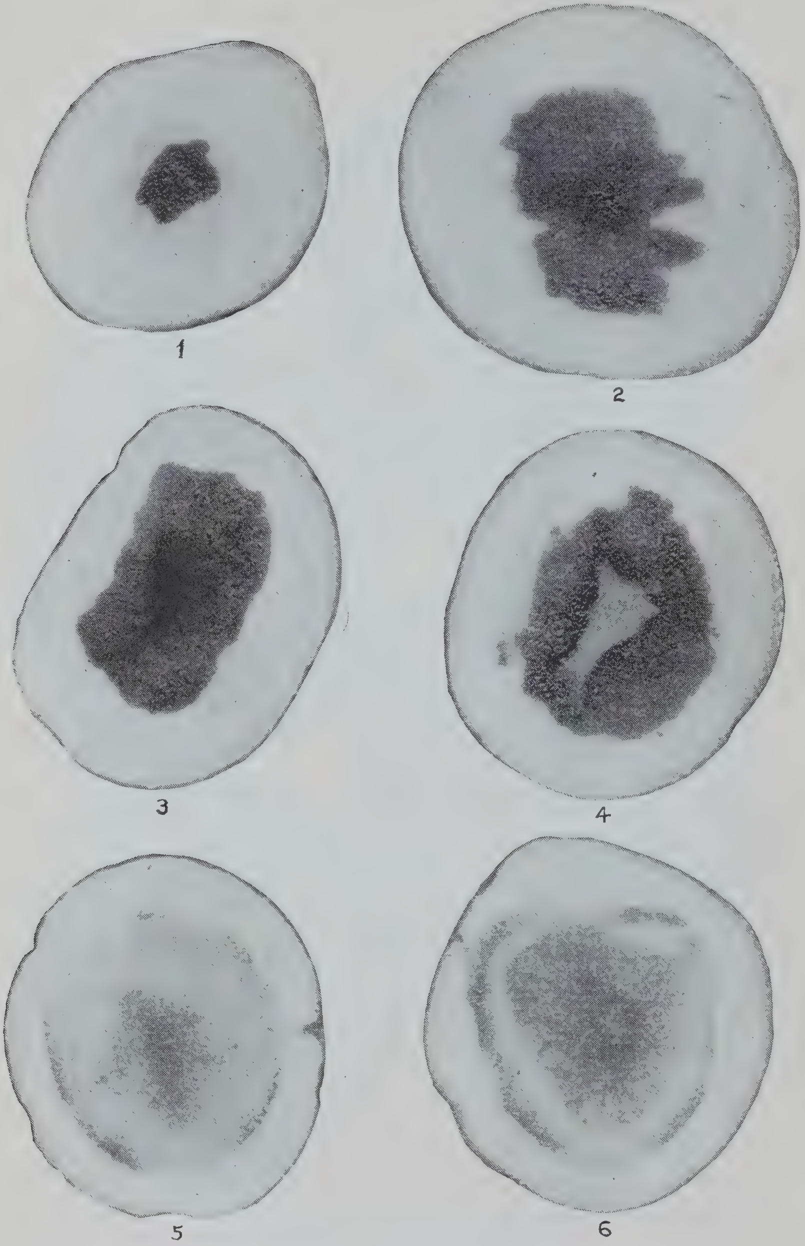


PLATE XII.— BLACKHEART OF POTATOES CAUSED BY EXPOSURE TO HIGH TEMPERATURE.
(For explanation of plate, see page 307.)

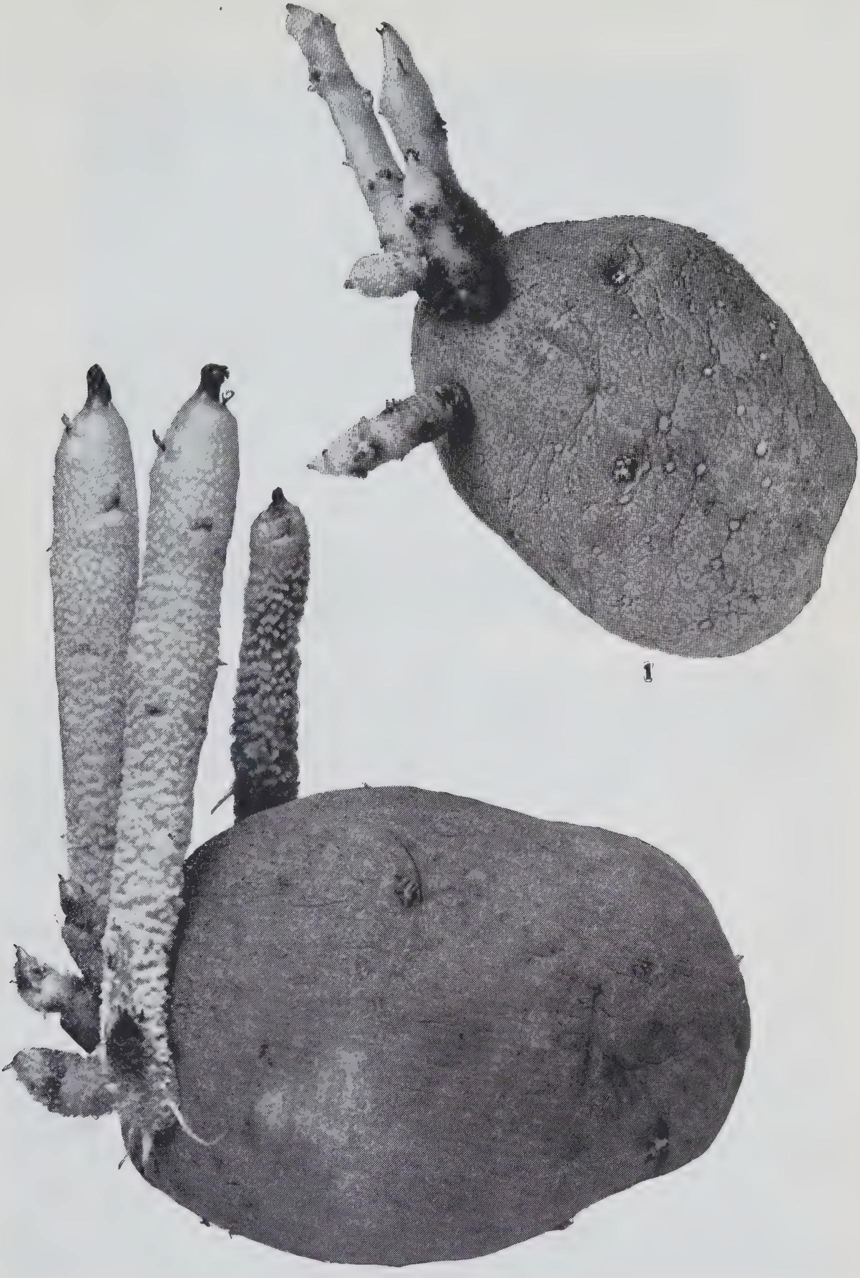
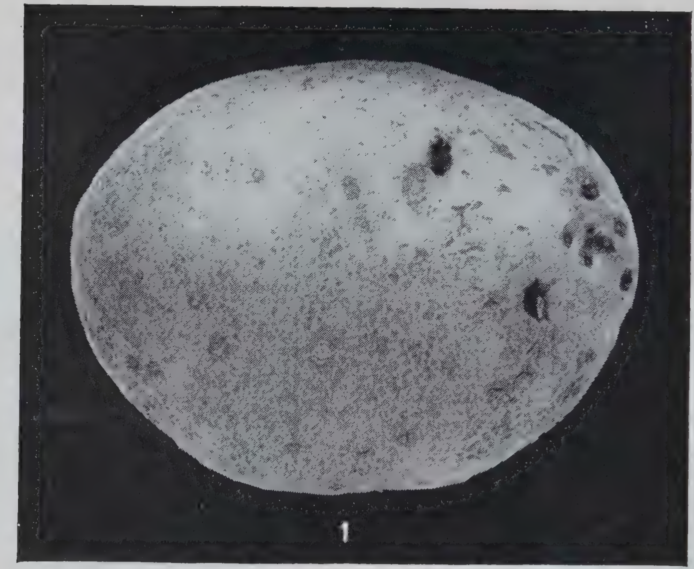


PLATE XIII.— CONDITION OF TUBERS IN FIRST TANK EXPERIMENT.
(For explanation of plate, see page 307.)



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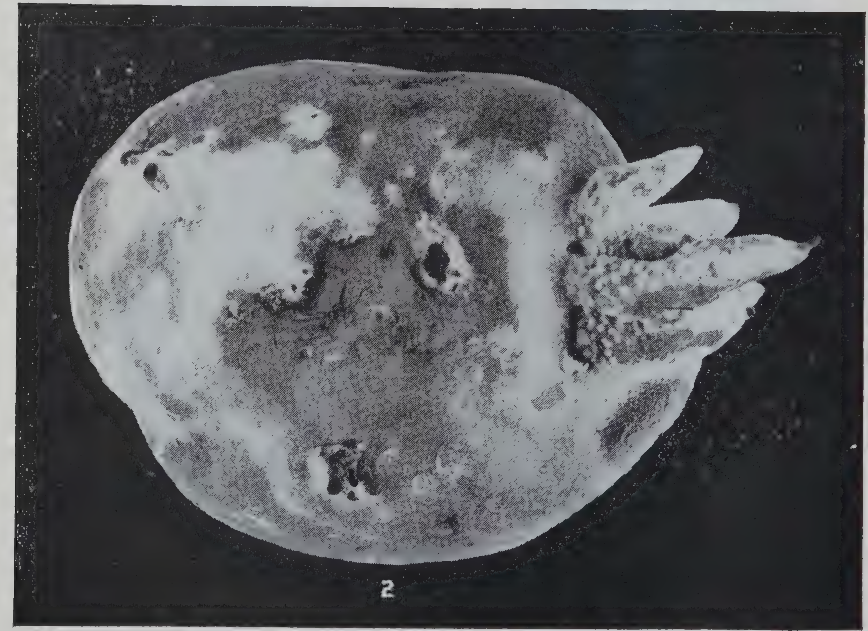
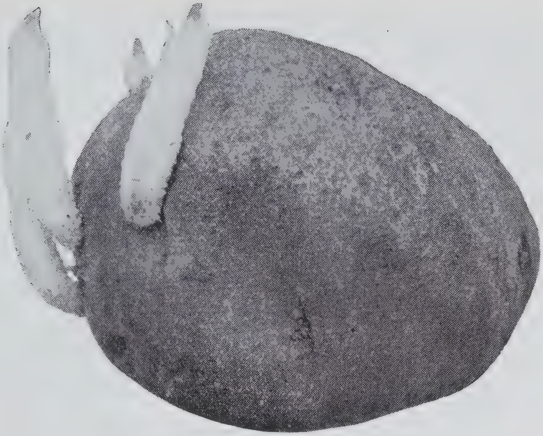


2

Plate XIV.— Condition of Tubers in First Tank Experiment.
(For explanation of plate, see page 307.)



1

2

Plate XV.—Imperfect Aeration Checks the Sprouting of Potatoes.
(For explanation of plate, see page 307.)

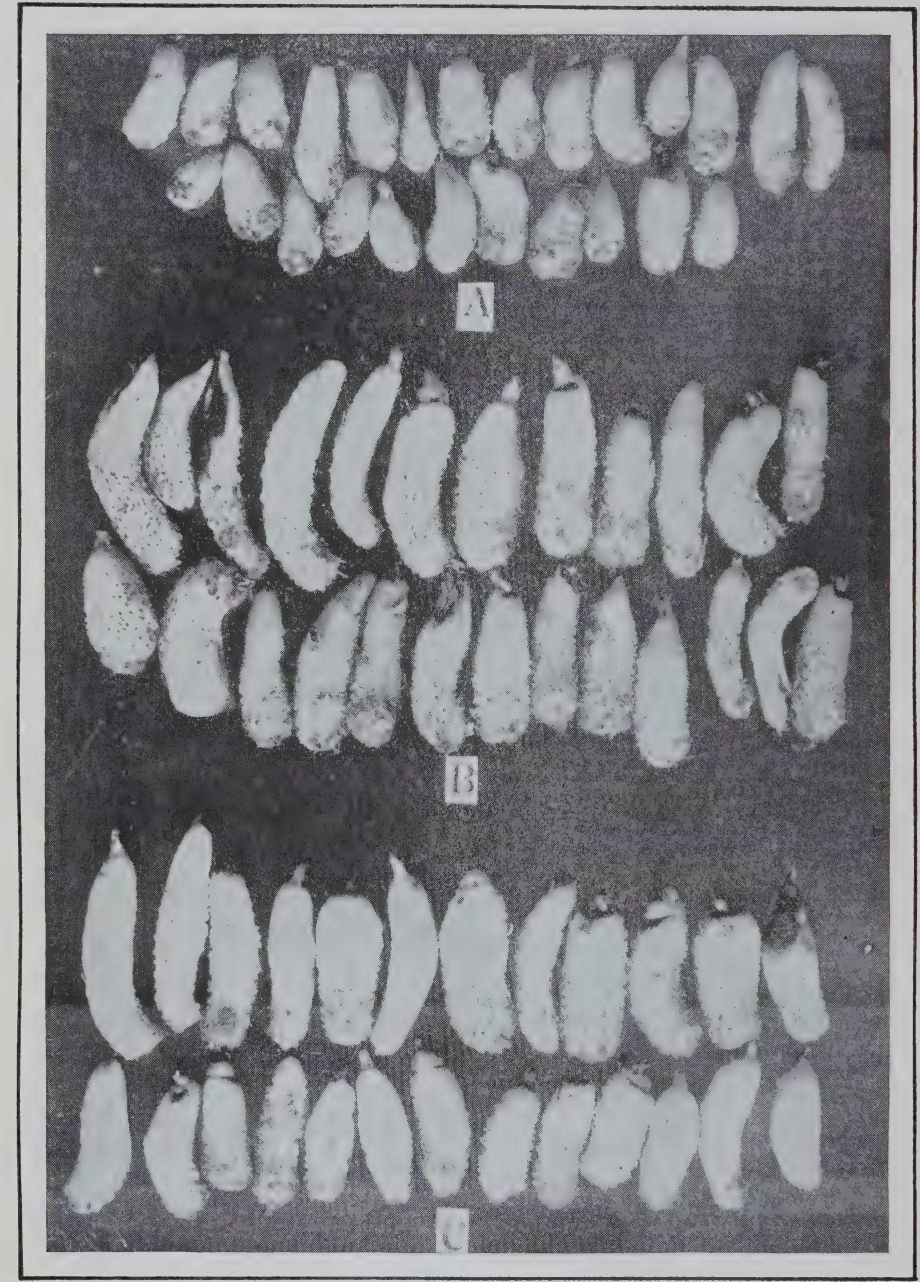


PLATE XVI.— SPROUTS FROM POTATOES IN FOURTH TANK EXPERIMENT.
(For explanation of plate, see page 308.)

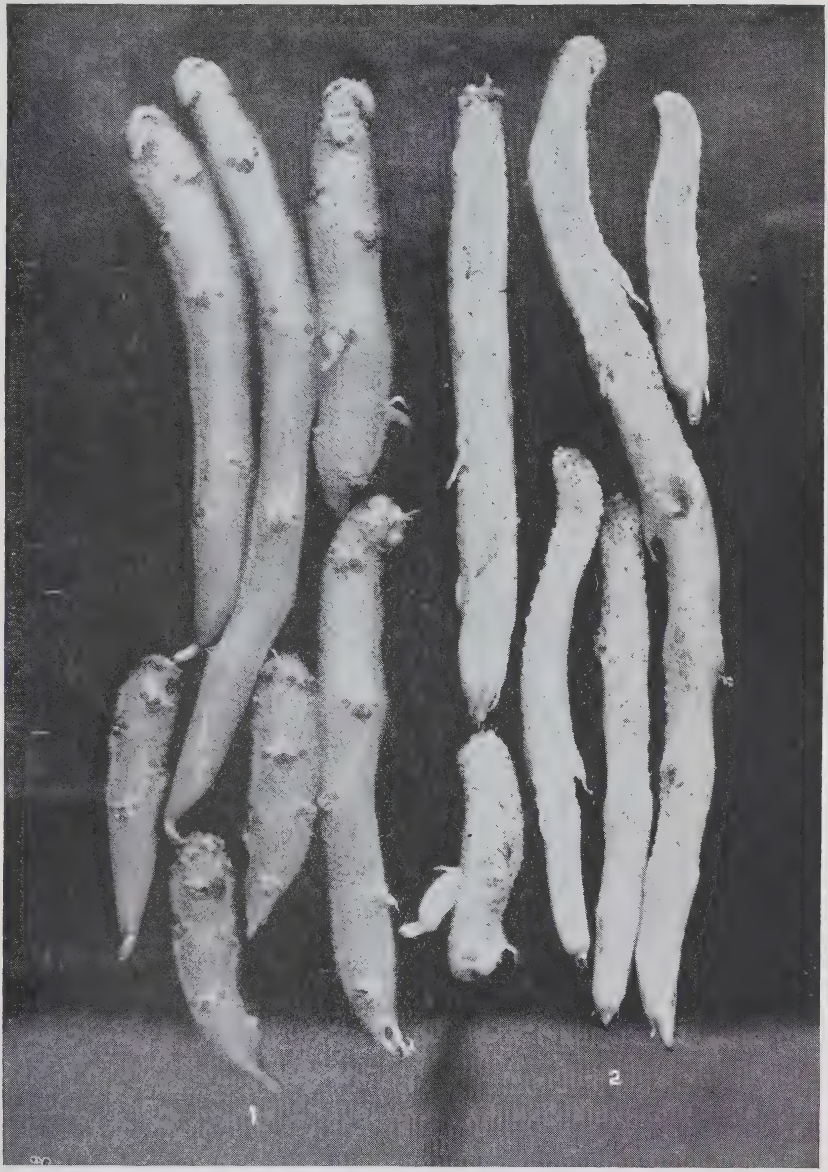


PLATE XVII.— SPROUTING OF POTATOES IN UPPER PART OF TANK C IN THE FIFTH TANK EXPERIMENT.

(For explanation of plate, see page 308.)

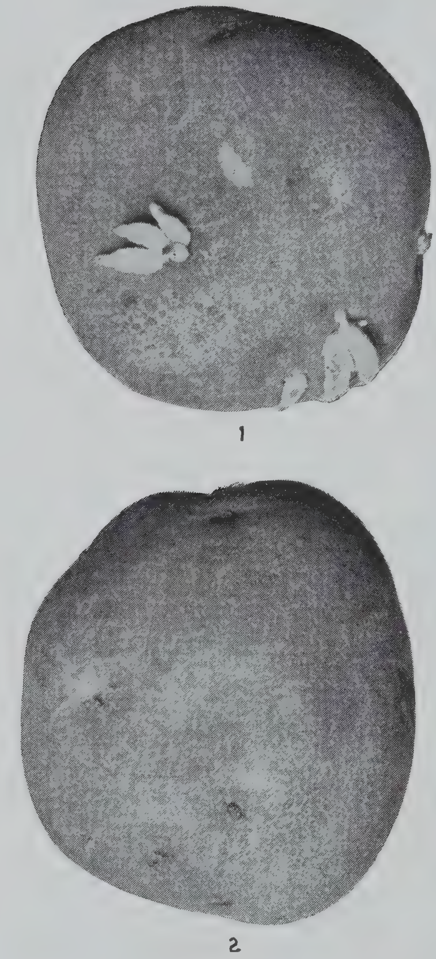


Plate XVIII.— Sprouting of Potatoes in Lower Part of Tank C in the Fifth Tank Experiment.
(For explanation of plate, see page 308)

heit thermometer was used to record the temperature. Readings were made at irregular intervals of a few days. The dates of reading and the temperature are given in Table IV.

The potatoes in Tank D were taken out on May 11, 1916, at the end of 185 days. The tubers on top had begun to sprout, but below a depth of 15 or 20 inches no sprouts had started. In Sections 2 and 3 the tubers were a little damp. None of the tubers anywhere in the tank were affected with mold, rot, blackheart, internal cavities or external discoloration. All appeared to be in perfect condition notwithstanding some of them had been confined at a depth of over six feet for six months at a temperature considerably above the freezing point most of the time.

Fifteen days later (May 26) Tanks E and F were opened. In both tanks the tubers on top had sprouted some, the majority of the sprouts being about one-fourth inch long. As in Tank D, no sprouts had started below a depth of about 15 inches. All tubers in Section 1 of both tanks were in good condition; also, a few at the top of Section 2; but a short distance down in Section 2 mold appeared, and in the greater part of this section the tubers were ruined by rotten spots apparently caused by a white mold which appeared in tufts, particularly at the eyes. In Section 3 of both tanks the tubers were all ruined with mold and rot. Twenty-five tubers from Section 3 of Tank F were cut at once. Large lenticular cavities were found in eleven of them, but no blackheart in any until after exposure to the air, when much blackheart appeared. In Section 2 of both tanks a considerable number of tubers developed blackheart upon exposure to the air. No blackheart or cavities were found in Section 1.

From an examination of Table IV it will be seen that up to May 11 the highest temperature recorded was 48.5° F. It is probable that this was the highest temperature during the period. Unfortunately, only two readings were made between May 11 and May 26. However, there is good reason to believe that 49° F., recorded on May 19, was the highest temperature up to that date; and 52° F., recorded on May 26, the maximum temperature for the entire period.

Altho, apparently, the temperature was above 40° F. during about one-half of the period of 185 days during which the tubers in Tank D were confined, they came out in perfect condition; but in Tanks E and F an additional period of confinement of fifteen days at a temperature of 48.5–52° F. resulted in the ruin of most of the tubers below a depth of thirty inches. This indicates that it is safe to store potatoes in piles six feet deep for at least six months provided the temperature is kept below 40° F.; but that great risk is incurred by extending the storage period even a few days beyond six months with the temperature above 50° F.

The appearance of blackheart in this experiment at a depth of three to six feet shows that blackheart may occur in common cellar storage at a temperature of 52° F. (11° C.).

STORAGE OF POTATOES IN UNVENTILATED PITS.

Frequently, potatoes are stored out-of-doors in piles which are protected from freezing by covering them with straw and soil or coarse stable manure. Piles of potatoes so protected are called pits. If the pits are large it is customary to provide ventilation by means of tubes or pipes; but in small pits the potatoes are completely enclosed by the protective covering of straw and soil.

Upon coming to a realization of the fact that potatoes require a large amount of air the writers began an inquiry into the condition of potatoes in unventilated pits. Undoubtedly, potatoes are often stored successfully in unventilated pits. Several potato growers have assured us that they have stored potatoes in this manner for several years in succession without mishap. On the other hand, complaints of the decay of potatoes in pits, both ventilated and unventilated, are not infrequent.

One such complaint addressed to the Station in 1907 is of special interest. A gardener at Glen Cove, Long Island, sent ten tubers accompanied by the following statement: "I am sending herewith some potatoes affected by a disease which is new to me and my gardening friends. The potatoes were dug as usual and pitted in the garden in the fall. When the pit was opened in early May 75 per ct. could not be used, the outward sign being no growth and dark appearance."

The senior author studied the potatoes and made the following note upon them: "Upon cutting the tubers two were found to be sound while each of the other eight had a large cavity surrounded by a wide zone of blackened tissue. The affected tissue was deep black and rubber-like. Some of the spots had connection with the surface; for example, one had a fork hole and another had connection with dry, rotten spots on the surface. However, in two tubers which were carefully examined there was no connection between the cavity and the surface. In these, the blackened tissue was free from fungus hyphae and bacteria so far as could be determined by microscopic examination."

The nature of this potato trouble was long a mystery; but it is now clear that it was a case of blackheart due to lack of air.

Another noteworthy occurrence of blackheart among potatoes stored in a pit is that recorded by Güssow.[11] This happened at Ottawa, Canada, on the Central Experimental Farm. Some potatoes stored in a pit were severely affected with blackheart while others of the same lot stored in a root cellar and a heated barn were unaffected. The temperature of the pit was taken daily by means of a self-recording thermometer located near the center of the pit. The highest temperature recorded was 46.5° F.; the lowest, 32.5° F. When the pit was opened it was found that some of the tubers on top had frozen. Since the highest temperature in the pit was far below that required for blackheart (according to Bartholomew) it was necessary to account for the occurrence of blackheart in some other way than by high temperature. In the root cellar and barn, where the potatoes were not affected, the temperature had not fallen below the freezing point; while in the pit some of the tubers had certainly been subjected to freezing temperature. Observing this and lacking a better theory Güssow was disposed to regard frost injury as the cause of the blackheart.

Notwithstanding it is distinctly stated by Güssow that " good ventilation existed in all three places of storage " the writers are confident that the potatoes in the pit lacked air and that the blackheart was due to asphyxiation.

Thru the kindness of Messrs. B. E. Smalley of Interlaken and J. Q. Wells of Shortsville the writers have had an opportunity to examine tubers stored in unventilated pits during the winter of 1914–1915. In both cases the tubers, which were unsprouted at the time of removal from the pit, afterward sprouted normally and were unaffected by blackheart.

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[11] Güssow, H. T. Report of the Division of Botany. Canada Expt. Farms Rpts. 1914:831–849. 1915.

AN EXPERIMENT WITH POTATOES IN UNVENTILATED PITS.

During the winter of 1915–1916 the writers conducted an experiment with potatoes in unventilated pits. Three pits containing ten bushels each were used. On November 15 the potatoes were placed in conical piles and covered first with a layer of oat straw and then a layer of clay loam soil. In the center of each pile there was placed a glass tube one-half inch in diameter. The lower end of this tube was about four inches above the bottom of the pile and its upper end projected about two feet above the top of the pile. In each tube a thermometer attached to a string was lowered to the bottom. The tops of the tubes were closed with rubber stoppers.

On December 1, after the first layer of soil had become frozen, a second layer of straw was applied and over this a second layer of soil. The covering of the three piles was made as nearly uniform as possible and no thicker than necessary to protect the potatoes from freezing. It was planned to apply an additional covering of coarse stable manure in case the weather became very cold, but the winter being a mild one this was not needed.

At the time of the second covering two of the tubes were accidentally broken. Hence, it was possible to obtain the temperature in only one of the pits. At irregular intervals of a few days the thermometer in this pit was drawn up and read. The temperature of the outside air was recorded at the same time. (See Table IV.) During February and March the pits were deeply covered with snow most of the time.

On May 5 one of the pits was opened. The tubers were wet all over. Those at the top had sprouts one-fourth inch long, while in the bottom of the pile sprouting had barely begun. All of the tubers were in good condition — free from blackheart and external blemishes.

Another pit was opened on May 26 and the third on May 27. In both pits the tubers were sprouting vigorously. Tubers on the surface had sprouts 2–4 inches long; those on the interior 1–2 inches long. Occasional sprouts were decayed, but the great majority were in good condition. The tubers themselves were sound and normal. Very few had external blemishes and none showed the least sign of blackheart or cavity formation internally.

The excellent condition of the tubers in the last two pits was surprising. Having previously opened Tanks E and F of the seventh tank experiment and found a large part of their contents ruined by blackheart and decay (page 353) the writers expected to find the potatoes in the pits in as bad or worse condition. This was expected because the potatoes in the pits had been confined almost as long as those in the tanks and the temperature of the pits had been slightly higher than that of the tanks most of the time. That the temperature of the pits was actually higher than that of the tanks, as indicated by the temperature record (Table IV), is proven by the greater length of the sprouts in the pits. They were 2–4 inches long, while those in the tanks were only one-fourth inch long. The potatoes were of the same variety, grown in the same lot and similarly treated up to the time of beginning the experiments except that the tubers used in the tanks were washed, while those used in the pits were not washed.

Thus, it appears that the tubers in these small, unventilated pits were better aerated than tubers at a depth of three to six feet in tanks open at the top. It seems impossible, but it must be true. Had the pits contained larger quantities of tubers or been more deeply covered the results might have been different.

IS THE INJURY DUE TO THE ABSENCE OF OXYGEN OR TO AN EXCESS OF CARBON DIOXID?

Respiring potato tubers consume oxygen and give off carbon dioxid. Carbon dioxid being slightly heavier than air has a tendency to settle. Hence, it is probable that a considerable quantity of carbon dioxid accumulated in the lower portions of the galvanized-iron tanks used in the tank experiments. Certainly, in sealed containers, the carbon dioxid produced remains in contact with the tubers, because it cannot escape. Now, the question arises: May it not be the accumulation of carbon dioxid rather than the absence of oxygen which causes the discoloration, blackheart and death of tubers suffering from insufficient aeration?

An attempt was made to obtain an answer to this question from experiments with tubers in sealed containers so arranged that the carbon dioxid evolved was absorbed as fast as produced. Two such experiments were made — one in February, 1915, the other in March, 1916.

In the 1915 experiment two glass jars were used. First, 250 cc. of a 40 per ct. solution of sodium hydroxid was placed in the bottom of one jar and an equal quantity of distilled water in the other. Next, a wire-cloth support to keep the potatoes from touching the liquid was put into each jar, and on top of this twenty-five tubers weighing fifty-six ounces. Finally, the jars were sealed with melted paraffin. They were held in this condition fourteen days at a temperature of 14–21° C.

A heavy, white precipitate (afterward tested with HCl and proven to be sodium carbonate) formed in the bottom and also on the surface of the sodium hydroxid solution. The jar was shaken once a day to keep the surface layer of this precipitate broken up.

When the jars were opened at the end of fourteen days the tubers in the one containing sodium hydroxid solution were nearly free from moisture and a few had a slightly wilted appearance, while those in the other jar were all moist over their entire surface. No sprouts had started in either jar. Upon exposure to the air both lots of tubers soon became discolored in the usual manner, those from the sodium hydroxid jar being distinctly more severely affected than those from the other jar. Eight days after removal from the jars the tubers were cut and examined for blackheart. Those from the sodium hydroxid jar showed 100 per ct. affected with blackheart; those from the jar containing distilled water, 64 per ct.

In the second experiment the containers used were a galvanized-iron cylinder having a capacity of about seven quarts and a glass jar holding about 3.5 quarts. Both were provided with tight-fitting covers. Two hundred and fifty cc. of a 33 per ct. solution of sodium hydroxid were put into the cylinder, and 250 cc. of distilled water into the glass jar. Twenty-nine tubers weighing 100 ounces were put into the cylinder over the sodium hydroxid, and sixteen tubers weighing 53.5 ounces into the glass jar over the distilled water. Wire-cloth supports were used to keep the potatoes up out of the liquids, as in the preceding experiment. Both the cylinder and the jar were made air-tight with paraffin and then exposed to a temperature of 15–21° C. for thirteen days.

All sixteen of the tubers in the glass jar were more or less moist on the surface. Six were moist over about one-third of their surface, while the remaining ten had moist areas of less extent. After a few hours' exposure to the air all showed the usual discolored areas of various sizes, in some cases as much as one-third of the surface being involved. All were affected with blackheart.

One tuber in the cylinder containing sodium hydroxid solution had a moist area covering one-third of its surface, but the other twenty-eight were dry. Most of the tubers were considerably shriveled and pitted at the bud end when first removed from the cylinder. Upon exposure to the air the entire bud end turned brown, giving the appearance (on the surface) of an attack of late-blight rot. All were affected with blackheart quite as severely as the tubers over distilled water. In this experiment no precipitate formed in the sodium hydroxid solution.

It is believed that in both experiments the quantity of sodium hydroxid solution was abundantly ample to absorb all of the carbon dioxid given off. In the second experiment it must have been more than four times as large as required. Yet the severity of the discoloration and blackheart was not lessened. This seems to prove that it is not carbon dioxid which causes the injury.

The cause of the peculiar shriveling and pitting of the tubers over sodium hydroxid solution in the second experiment was not determined.

BLACKHEART ALSO A SHIPPING PROBLEM.

Bartholomew has pointed out that blackheart sometimes results from the overheating of potatoes during shipment in stove-heated cars. Recently, an instance of this kind came to the attention of the writers.

In February, 1917, the Station received a complaint concerning blackheart among a lot of seed potatoes shipped from Clinton county, N. Y., to Peconic, Long Island. A few sample tubers which accompanied the complaint appeared nearly or quite normal externally, but had large areas of blackheart within. It was stated that about 25 per ct. of the tubers in a shipment of eighty sacks (220 bushels) were similarly affected. In some sacks as much as 90 per ct. of the tubers were affected while other sacks were entirely free from the trouble.

The sender was informed that the symptoms indicated that the tubers had been overheated. Upon being requested to furnish information concerning the treatment of the potatoes during shipment he replied that the sacks of potatoes were packed in saw-mill refuse in a car provided with air chambers on the sides and bottom. He stated that inquiry at the shipping point had elicited the information that after the car was loaded the temperature suddenly dropped to —20° F. To prevent the potatoes from freezing two stoves were put into the car and the temperature kept at 120° F. for a week until the weather moderated. Then the stoves were removed and the car started on its journey.

In the light of Bartholomew's experiments it is clear that the blackheart of these potatoes was due to the high temperature to which they had been subjected in the car.

However, it is equally clear that many of the tubers did not experience the high temperature indicated. This is shown by the fact that many escaped injury. A temperature of 120° F. maintained for a week would have ruined every tuber subjected to it.

The shipment of potatoes in stove-heated cars appears to be a rather common practice. Shippers should understand that it is attended with risk. Temperatures above 100° F. may be quite as injurious as a freezing temperature.

BLACKHEART PRODUCED AT HIGH TEMPERATURES VS. BLACKHEART PRODUCED BY EXCLUSION OF THE AIR.

Blackheart may be produced in different ways by varying the three factors involved, viz., the temperature, the oxygen supply and the length of the exposure.

In this study the writers have focused their attention upon the oxygen factor and employed low temperatures (below 75° F.) in most of their experiments. Only one series of experiments was made with tubers in an incubator at a temperature above 100° F. The blackheart so produced (Plate XII) was like that described by Bartholomew and resembled the blackheart produced in our own experiments by exclusion of the air at lower temperatures.

However, there appears to be one character by means of which it is possible to determine whether a given case of blackheart was brought about by exposure to a high temperature for a short time in the presence of an abundant supply of air, or by the exclusion of the air for a long time at a low temperature. Tubers affected with blackheart produced by high temperature usually appear normal externally,[12] while those affected with blackheart produced by exclusion of the air usually show more or less surface discoloration. (Plate I.)

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[12] Bartholomew, *loc. cit.*, p. 637.

RELATION OF BLACKHEART TO SEED POTATOES.

When seed potatoes show blackheart the question arises, How does it affect their value for planting? Also, is it safe to plant unaffected tubers from the same lot?

Comparative planting tests have not been made, but observations on the sprouting of tubers affected with blackheart leads to the belief that only severely affected tubers need be rejected. The sprouts of blackhearted potatoes appear to be normally robust and vigorous.

If the blackheart is accompanied by the discoloration of a considerable portion of the surface the affected tubers should not be used for planting. Eyes on the discolored areas will not sprout and such tubers are liable to decay quickly. Probably, it is advisable to reject, also, all tubers having large areas of blackheart, even tho normal externally. But tubers free from external discoloration and showing only small to medium-sized areas of blackheart may be planted. As for associated tubers which show neither blackheart nor external blemishes, there is no reason to suspect that they are in any way injured for seed purposes. If seed potatoes are sound and normal in appearance it is unlikely that they have been injured by any condition of storage to which they may have been subjected.

APPLICATION OF THE RESULTS TO THE PROBLEM OF POTATO STORAGE.

The results of this investigation emphasize the importance of providing ventilation for potatoes in storage. The need of ventilation depends very largely upon the temperature. As the temperature rises the volume of air required increases rapidly. At low temperatures, potatoes may be stored in deep piles for long periods of time. At high temperatures, it is necessary to avoid deep piling or else provide special means of ventilation. If the temperature is kept below 40° F. potatoes may be piled in bins and cellars to a depth of six feet without any ventilation except that provided thru free access to the air overhead. Under such conditions, potatoes may be stored with safety for at least six months and perhaps longer. It is probable that no harm will result if the temperature goes up to 45° F. for a few days. But a long period of storage followed by a two weeks' exposure to a temperature of 50° F. or higher is likely to result in the ruin of most of the tubers below a depth of about three feet. Potatoes stored in deep piles should be carefully watched in the spring as the temperature rises. A few days of high temperature may cause much loss.

It is not in the province of this bulletin to discuss the means by which the ventilation of potatoes may be secured. The methods to be employed will vary according to circumstances. The principles to be kept in mind are as follows:

(1) Potatoes stored at high temperatures require more ventilation than those stored at low temperatures.

(2) Better ventilation is required for potatoes which are to be stored for a long period than for those which are to be stored for only a short time.

(3) Until more accurate determinations are made, six feet should be regarded as the maximum depth to which potatoes may be piled without special provision for ventilation when stored for six months at temperatures below 45° F. If greater depths than six feet are employed ventilators should be provided and so arranged that none of the tubers will be more than six feet distant from an abundant supply of air.

(4) At temperatures of 50–70° F. potatoes should not be piled over three feet deep if they are to be kept longer than about three weeks.

(5) No kind of ventilation is sufficient to prevent the occurrence of blackheart in potatoes kept for even a few days continuously at a temperature above 100° F.

(6) Complete exclusion of the air will ruin potatoes at any temperature.

(7) Small potato pits do not need ventilation; but some provision should be made for the ventilation of large pits.

NECK-ROT DISEASE OF ONIONS.[1]*

M. T. MUNN.[2]

SUMMARY.

The neck-rot disease of onions apparently occurs in every onion-growing district in Michigan and New York and in every principal onion section in the United States. It causes heavy losses to stored onions and frequently to the growing crop and the seed crop. Evidence has been secured that the disease is destructive in Europe. The most striking signs of the disease are to be found in the storage houses where the causal fungus appears as masses of sclerotia at the necks of the onions. However, these may cover part, or all, of the individual bulbs. The smoke gray covering of fungous growth commonly recognized by onion growers as "gray mold" is another striking sign of the disease.

The cause of the disease as determined by numerous inoculation experiments is a fungus which has been described and given the name ***Botrytis allii.*** Infection of the bulbs in the field occurs thru the leaves and necks. The fungus passes down the necks and causes a neck rot in the field, or else it goes into the storage house in the form of mycelium in the necks and bulbs where the disease develops rapidly under favorable conditions. The bulbs may also become infected from the soil. The seed

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heads become infected by wind-blown spores, causing a blasting of the flowers.

The fungus apparently produces appreciable amounts of oxalic acid. However, it was not possible to demonstrate that this acid produces the pathological changes in tissue observed when the fungus attacked the host. It was found that the fungus secretes the enzym pectinase. This enzym was precipitated and re-dissolved from the fungal extract. From a study of the action of the fungal extract upon plant tissue discs it was possible to explain all of the tissue changes taking place when the parasite attacks the host.

Factors favorable to infection and the occurrence of the neck rot were found to be as follows:— (1) Immaturity and imperfect curing of the bulbs; (2) the application of commercial fertilizers late in the season or in incorrect proportions; (3) the application of large quantities of stable manure before planting; (4) poor air drainage in the onion fields; (5) high humidity, high temperature and poor ventilation in the storage house.

Methods of control, so far as worked out, lie mainly along the line of field sanitation, care of the curing crop, and storing in properly constructed and regulated houses. Diseased bulbs should not be used as " mother bulbs " for producing the seed crop since they are an important source of infection to the seed heads, and later, to the growing crop in nearby fields. Piles of decaying and refuse onions should not be permitted near the onion fields or used for fertilizer thereon. Care should be exercised in handling the crop to prevent unnecessary bruising on the topping machine and while handling the stock to be stored. The crop should mature and die early. Remove all refuse tops, soil and screenings. Cure thoroly in the field or in the drying sheds. Store the crop in slatted crates in well constructed houses having ample facilities for ventilation and the maintenance of low temperature.

Fumigation of the stock with formaldehyde gas either just before or after storing has been found to be both ineffective against the fungus and impracticable in the average storage house. While spraying the growing crop with bordeaux mixture has given some promising results, it has not been tested sufficiently to warrant definite recommendations. Apparently, the spraying of onions has not proven popular with onion growers because of certain difficulties encountered.

INTRODUCTION.

HISTORY OF PRESENT INVESTIGATION.

The incident which, in part, initiated this investigation, was the receipt on February 24, 1913, of specimens of diseased onions from Mr. O. L. Humphrey, manager of the Upton Storage Company's houses at Honeoye Falls, N. Y. A letter accompanying the specimens stated that the onions in the storage house were decaying rapidly and that the loss would be complete unless measures were at once taken to stop the spread of the disease. At the suggestion of Mr. F. C. Stewart, Botanist of this Station, an investigation was started. Upon visiting this storage house 700 bushels of yellow onions grown upon muck land were found stored in a rather damp basement. All of the onions were badly diseased, nearly every bulb showing the presence of large masses of black sclerotia encircling the necks, with an occasional specimen showing a gray, dense, close-growing weft of a fungus readily recognized as a species of Botrytis. The loss was practically complete. A visit to other onion storage houses in central and western New York revealed the fact that considerable storage loss was taking place because of this onion disease. It was evident that we were dealing with a disease of great economic importance and one whose etiology was obscure and control measures unknown. Further investigations and studies, herewith reported, were made during the years 1913, 1914, 1915, and 1916 from the Geneva Station, and during the fall of 1915 and winter and spring of 1916 at the Michigan Agricultural College. In Michigan, as in New York, the onion growers and storage house owners and operators had invariably the same experiences to relate. Their crops were apparently well cured and sound when stored; but a short time after storage began, about October or later, the stock became badly rotted, the rot increasing directly as the storage season advanced. The stock was sorted, but unless the apparently sound onions were marketed immediately the crop soon became an almost complete loss to the owners. Unusual care in curing and storing the crop did not appear to prevent the disease.

ECONOMIC IMPORTANCE OF THE ONION.

The Thirteenth Census of the United States shows that 47,625 acres of onions, valued at $6,709,047 were produced in 1909, the onion holding third place among the truck crops of the United States. Every state is reported as producing some onions. Both as to acreage and value of onions produced, New York and Ohio were the leading states. In 1909 the largest onion acreage for any geographical division was reported from the East North Central States, including Ohio, Indiana, Illinois, Michigan and Wisconsin. The Middle Atlantic Division, including New York, New Jersey and Pennsylvania reported the second largest acreage. The writer feels confident that the onion acreage as reported by the census is low; at least this appears to be true for the states of Michigan and New York. According to Blair (1916) the area planted with onions in 1915 in the principal onion producing states was estimated by the Department of Agriculture to be 49,573 acres.

NEW YORK ONION DISTRICTS.

According to the Thirteenth Census of the United States, New York State in 1909 ranked second in onion production, having an acreage of 5,558 acres, valued at $954,610. Only seven counties in the State are reported as producing no onions. Orange County leads in onion production having approximately 2900 acres in the vicinity of Goshen, Florida, and Pine Island. Wayne County is second with 700 acres located near Rose, while Madison County is third with 570 acres in the Canastota region devoted to the production of this crop. The other important onion growing regions are in the counties of Queens, Monroe, Erie, and at South Lima in Livingston County.

MICHIGAN ONION DISTRICTS.

In Michigan the largest onion sections are in Allegan and Kent Counties. The Allegan acreage comprises the districts at Plainwell, Gun Marsh, Martin, Shelbyville and Wayland. The Kent County district is located between Byron Center and Ross. The other important districts are at Big Swamp in Lenawee County, Grant in Newago County, Zealand Township, Hudsonville and Vriesland in Ottawa County, Mentha, Van Buren County, and at Manchester in Washtenaw County. In the Thirteenth Census of the United States, Michigan is reported as having in 1909 an onion acreage of 1,130 acres, the crop being valued at $146,507.

THE DISEASE.

NAME.

There are a number of common names applied to this disease. The list comprises Mold, Gray Mold, Storage Rot, Rot, Onion Rot, Onion Bulb Rot, Dry Rot, Black-neck Rot, Dry Neck-rot, Stem Rot, and Neck Rot. Of these names the last two seem to be more descriptive of the disease. The writer prefers the name Neck Rot since it more accurately describes the appearance of the disease in storage, a form which the grower readily recognizes. Furthermore, the term stem cannot be used, botanically, for the parts of the host plant above the bulb usually attacked by the fungus. Accordingly, the name Neck Rot will be used in this account.

PLANTS AFFECTED.

The neck-rot disease caused by a parasitic Botrytis seems confined to *Allium cepa*, a broad species with many widely varying varieties. It has been found on perennial or tree onions, on seed onions, and growing or market crop onions. Schröter (1879) reported a Botrytis on *Allium ursinum* but gives no description whatever of the fungus. Frank (1896) stated that he also observed near Leipzig the Botrytis noted by Schröter.

DISTRIBUTION.

That the neck-rot disease of onions, or what appears to be this disease, is widely distributed is indicated by reports from nearly every onion growing region. Sorauer, as early as 1876, reported a Botrytis disease of onions from Germany, while a few years later Frank (1880) reported it from the same place. Bruck (1907) also mentioned this disease as being common in Europe. Voglino (1903) stated that he found what appears to be this disease in Italy and France in 1892. An onion disease caused by a Botrytis was noted in England in 1894 according to Massee. Halsted (1890) appears to have been the first in America to report a neck rot of onions due to a Botrytis. Clinton (1903) reported this disease from Connecticut; Jarvis (1909) also reported it from that state. The disease was recorded by Selby (1910) as being abundant in Ohio, while Jackson (1914) stated that it is a serious storage trouble of onions in Oregon. Humbert (1916) reported heavy storage losses from this disease upon white onions in Ohio districts.

In the fall of 1915 the writer noticed the neck-rot fungus upon some large white onions in crates in the Grand Trunk Railway freight house at Lansing, Michigan. These onions were consigned to a Lansing dealer and were shipped from Bermuda. Isolations in pure culture confirmed this observation. Again, in 1916 and 1917, the Botrytis neck-rot fungus was found upon, and isolated from, large white onions shipped from Bermuda and also from Texas.

In a letter from Dr. Johanna Westerdijk of Amsterdam, Holland, addressed to Professor H. H. Whetzel of Cornell University and dated January 4, 1917, an interesting reference is made to a disease of the onion. Dr. Westerdijk supplied Professor Whetzel with a culture of a Botrytis which she stated caused a severe disease of the leaves of onion. A critical study of transfers from the culture furnished to Professor Whetzel showed the fungus to be identical in every particular with the cultures of the onion Botrytis secured from various places in America. Dr. Westerdijk also stated that she had described the disease in a brief paper. Unfortunately, no further information regarding the disease could be obtained from this source at this time.

A report based upon the records[1] of the Plant Disease Survey (Bureau of Plant Industry) show that the Botrytis neck rot of onions has been reported by collaborators as being serious in the states of Connecticut, Illinois, Maryland, Massachusetts, Michigan, New York, Ohio, Oregon, Pennsylvania, Washington and Wisconsin.

ECONOMIC IMPORTANCE.

The margin of profit on storage onions is usually small, and unless a marked increase in price takes place a small amount of neck rot will offset the gain from storage. The neck-rot disease has practically prevented the growing of white onions in many onion sections in New York and Michigan. Figure 1, plate V, shows one of a number of white onions taken from an upland field early in the season of 1915. Their condition indicates the virulence of the fungus upon these tender, thin-skinned varieties.

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[1] These data are from unpublished records of the U. S. Dept. Agr. Bur. Plant Indus., Plant-Disease Survey. They include reports from states where *Sclerotium cepivorum* is believed to be the cause of the disease, also *Botrytis cinerea*, *Botrytis vulgaris*, *Sclerotinia fuckeliana*, *Sclerotinia* sp., and Sclerotinia (bulb rot) are reported as causing what is believed to be this disease of onions.

A study was made of the losses due to the neck-rot disease occurring in the retail trade of grocery stores dealing in southern onions. With the flat white onions from Texas a loss of about two per ct. occurred, this increasing slightly as the spring season closed. Frequently, one finds a badly diseased specimen among the large, white bulbs sold as Spanish and Bermuda onions. These are usually sold by the pound and the losses are correspondingly greater. The records of the Plant Disease Survey as furnished by collaborators in the states mentioned in the preceding section show that the losses vary from 7 or 8 per ct. to as high as 50 per ct. of the crop. In two instances it is reported that the acreage devoted to onion growing has been materially decreased because of the ravages of this disease. The highest losses are plainly associated with seasonal conditions which are termed " poor seasons for onions." Neck rot is plainly one of the limiting factors in onion growing.

ESTIMATE OF LOSSES IN MICHIGAN AND NEW YORK

For these states as a whole it is difficult to obtain any accurate data covering the losses in the yellow and red varieties of onions. The estimates must be limited to individual storage houses. During the storage season of 1912–13 the losses in some poorly ventilated houses were almost total, while in some houses of the better class the average loss was approximately one crate out of every ten at the time of sorting before shipment. During the storage season of 1915–16, following an exceedingly unfavorable growing season for onions, the storage losses were enormous, being practically total in many storage houses. In Michigan, early in the winter of 1915, the loss from neck rot in several storage houses was averaging one crate out of every six sorted, while by January 4 the losses were slightly over 50 per ct. of the total stored crop. Some definite, accurate data furnished by one prominent onion grower are as follows: Owing to a poor onion year this particular grower harvested about 25 per ct. of a crop. This gave 2200 bushels which were put in storage. The neck rot appeared in a severe form in less than six weeks after the stock was placed in storage. Of the 2200 bushels stored, 1200 were dumped out on the fields. The owners were able to realize upon but 1000 bushels. Of the entire crop only two bushels of bulbs were fit to save for producing a seed crop.

NATURE OF THE LOSSES.

To the growing crop.— The most important factor to be considered in this connection is the effect of the disease on the keeping quality of the bulbs. At the time of sorting, the neck-rotted bulbs are included with those attacked by the smut fungus, onion maggots, and all sprouted bulbs. This material is classed as " shrinkage " by the onion grower. A careful analysis of this " shrinkage " into its component parts showed it to be about 60 per ct. neck rot in some instances. An examination of many of the sprouted bulbs showed that the neck-rot fungus may have been the initial or exciting cause of the sprouting in many cases, since some moisture is present after rotting takes place. In one storage house under observation the loss due to the neck-rot fungus, six weeks after the crop was placed in storage, was approximately one crate out of every five — a " shrinkage " of about 20 per ct. In this particular house the loss was nearly complete by January 30.

Where the fungus attacks the bulbs thru the roots and the necks in the field the loss is due to a decrease in tonnage per acre because of a failure of the affected bulbs to produce size. The small-sized onions are classed as " seconds " and " picklers " and command a much lower price than the " primes."

Severe attacks of neck rot may follow frost injury to the crop early in the fall. Frosted onions are usually immature and bring considerable moisture into the storage house.

To the seed crop.— It is impossible to obtain accurate data as to the losses occurring in the seed crop since it is difficult to separate two factors which cause a failure of the seed crop, viz.: (1) Excessive moisture, which often prevents a setting of the seed heads; and (2) attacks of the neck-rot fungus, which also cause a failure of the flower heads to produce seed. Apparently, the two factors are inseparable, i. e., an attack of the fungus follows a period of excessive moisture. There is a so-called diseased condition brought about by excessive drought at the time of seed formation which is known to the onion seed growers as " blast." This condition is to be differentiated from the blasting of the seed heads due to the neck-rot fungus.

A general failure of the onion seed crop in certain regions in Michigan in 1915, and in some sections of New York in 1913 is believed by the writer to be due to the attacks of this fungus. Clinton (1903,

1914) reported the Botrytis fungus as causing a yellowing of the leaves and a blasting of the onion flowers early in the season in Connecticut. Here it was reported as doing considerable injury to the flowers in moist seasons.

SIGNS OF THE DISEASE.

ON THE LEAVES.

Where infection has taken place on the leaves thru the germination of conidia one finds at first small white spots which later enlarge. The diseased spots are very frequently elongated in the same direction as the cells of the host plant. The spots may be surrounded by a yellowish area of tissue having a water-soaked appearance. This is to be distinguished from a so-called physiological disease of onion leaves known as White Tip. In this the tips of the leaves turn white, whereas the neck rot fungus causes a slight yellowing of the leaves. Sometimes infection may occur where the leaves are folded over or following severe injury by thrips. Shortly following infection, in any case, the tissue adjoining the point of infection takes on a water-soaked or boiled appearance, the green color of the leaf disappears and is replaced by a yellowish brown. Clinton (1903) reported that he found the fungus (an onion Botrytis) appearing as a parasite on the green leaves comparatively early in the season.

ON THE NECKS.[1]

The disease occurs on the necks in the form of tufts of conidiophores bearing smoke-gray conidia (Plate XXIII, figs. 2, 3). The fungus may be readily found upon the necks of the " scallions," " bullnecks " or " thicknecks" which are put in storage. On these the fungus readily shows itself because the bulbs are not dry or mature when they go into the storage house. From the neck and outer leaves the fungus works down into the bulbs killing the tissue of the host in advance of the mycelium.

ON THE BULBS.

The neck rot is usually first brought to the notice of the onion grower by the appearance of roundish black bodies, or sclerotia, upon the onion bulbs in storage. The sclerotia are more frequently found at the necks of the bulbs occurring either singly or in the form of sclerotial crusts which may encircle the entire neck of the bulb (Plate XXIV, fig. 3). These sclerotial crusts at the necks may be pulled off rather easily, carrying with them the remains of the outer two or three bulb scales, or thickened bases of leaves, from which they are inseparable. These sclerotia may also be found upon the sides or bottoms of the bulbs, and often one finds a specimen with the entire side covered by a dense sclerotial crust (Plate XXIV, fig. 4).

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[1] The term neck as used in this paper is applied to that part of the onion plant just above the bulb, which resembles a stem, namely, the leaves which are enclosed in the sheath of the outermost leaf and extending well down to a point where the leaves become markedly thickened forming a bulb.

Occasionally, a bulb will be found which shows the presence of the sclerotia between the scales. The bulb may be very soft, contain considerable liquid and give off an offensive odor. In such cases it is believed that soft-rot bacteria follow the invasion of the fungus as secondary rot producers. As a rule but very little moisture is formed following the attacks of the fungus. It is because of this fact that this disease has been called dry rot and dry neck rot by some.

Some bulbs, especially those from the dry locations in the storage houses, will show a smoke-gray covering of the conidia and conidiophores of the fungus in the region of the neck. This form of the fungus is very frequently found on the flat white onions shipped to the northern states from the southern onion growing regions. Other bulbs may show the presence of both the sclerotial and the conidial stage of the fungus at the same time. Upon cutting into the diseased bulbs one frequently finds the spaces between the scales filled with a mat of mycelium varying in color from a dirty white to a dark brown. On cutting open bulbs which show only a few tufts of conidiophores at the neck, the bulb scales are nearly always found to be softened and discolored in the region of the neck and may show a mat of mycelium present.

ON THE ROOTS.

The fungus has been found causing a serious root rot of the bulbs in the field. The condition brought about by the root rotting is very noticeable later when the bulbs are put in storage where the fungus appears in the form of either sclerotia or tufts of conidiophores at the bottom end of the bulb, and also by the fact that the bulbs are considerably smaller than the average run of stock. When the sclerotia are formed at the bottom end of the bulbs they are frequently in the form of a ring or crust surrounding the base of the onion. Data obtained from storage inspections show that there was more root or bottom infection during the year 1915 than in any one of the previous three seasons. The ratio of neck to bottom infections as shown in some cases in 1915 was about as 13 to 8.

ON THE SEED HEADS.

The onion Botrytis has been found fruiting on the dead leaves, scapose stems, and the base of the seed stalks in a number of onion seed fields visited. In several instances the growers complained that the seed heads had been blasted. A careful examination of these heads showed that invariably the seed stalk, or seed head, or both, had been attacked by the neck-rot fungus. The heads showed the coarse, branching mycelium of the fungus upon and in the dead floral parts. Isolations showed it to be the onion Botrytis. A species of Cladosporium was found quite frequently upon the dead seed heads but inoculation experiments, reported elsewhere in this paper, showed the Botrytis to be the cause of the blighting or blasting of the seed heads attacked. At the base of the seed stalks where the fungus attacked the leaves the disease appeared to be more prominent where the leaf sheath clasped the stem and on old remnants of leaves adhering to the stem or seed stalk. Fig. 1, plate XXVI, shows two diseased and one healthy, normal seed heads. The head partly open shows the fungus fruiting just beneath the split in the membrane enclosing the flowers. The diseased seed head which is open shows the fungus fruiting upon the flower pedicels. Fig. 2, plate XXVI, shows a seed head which has been attacked by the neck-rot fungus (blasted). The black fungus bodies on the stalk are due to the presence of a species of Pleospora which appears after the seed stalks are dead.

ETIOLOGY.

INFECTION OF DRY BULBS BY DUSTING WITH DRY SPORES

During the work of going over hundreds of crates of red and yellow onions stored in various storage houses and in which the Botrytis fungus was constantly associated with the disease, in but very few instances was evidence obtained that the fungus passed from onion to onion except where the bulbs had been wounded either during harvesting or storing, or by being bruised while screening. The dry skins or scales surrounding the healthy bulbs appear to be a barrier to infection. However, it must be conceded that infection of healthy bulbs may take place where they are covered by a mass of wet, sprouting and rotting specimens. In order to test experimentally the possibility of infection thru dry skins, large sound onions were cleaned aseptically, by carefully removing the outer dry scales with sterile forceps, then placed on crystallizing dishes in deep preservation jars kept moist by the use of wet cotton in the bottom of the jars. Some of the bulbs had one of the outer dry skins intact while on others these were removed, exposing the fresh fleshy scales. These onions were divided into two lots; on some of each lot spores in sterile distilled water were placed upon the fleshy and the dry scales, while on others the dry spores were dusted upon the scales. Suitable numbers of each lot were left untreated as checks. Upon examination later it was found that none of the dry spores placed upon the dry, firm, fleshy scales had germinated; but in the cases in which the spores were suspended in drops of water, germination had taken place on both the dry skins and on the fleshy scales. Infection of the tissue followed on only a few of the fleshy scales. The extent of infection of the fleshy scales was especially marked where a small wound was present. The mycelium penetrated thru the fleshy scales discoloring the tissue and killing the cells. Harter (1916) reports that in his study of the storage rots of economic aroids it was found necessary to apply sterile water once or twice to the tubers and corms after inoculation with the fungi studied, but that no further applications of water were necessary after decay had once started. It was plainly evident from these experiments that drops of water, or sufficient moisture, must be present to bring about germination of the spores, and later, infection of the bulbs.

INFECTION OF GROWING PLANTS BY MEANS OF DRY SPORES.

All attempts to bring about infection of normal green and uninjured leaves and necks of onion plants by dusting dry spores over them were unsuccessful except when small drops of water were supplied, and the plants covered with bell jars for 36 hours. On very sunshiny days when only a small amount of moisture was supplied infection did not follow. In other words, infection from spores was successful only when the surrounding conditions were very similar to those which obtain in the onion fields when the plants are wet with dew or rain for two or three days and cloudy weather prevails.

INFECTION OF GROWING PLANTS BY MEANS OF SPORE SUSPENSIONS.

On July 8, 1915, twenty-three onion plants, started from white onion sets and growing in sterilized soil in three-inch pots, were placed in a large inoculation chamber. These plants were then thoroly atomized with a suspension of the onion Botrytis spores in sterile water. After inoculating the plants in the chamber, three additional plants which had not been atomized with spores were added to the lot to serve as checks. On July 12 every inoculated plant showed infection from the Botrytis on some of its parts. A number of the leaf tips were dead and showed abundant infection when examined a few days later. Every inoculated plant showed the fungus fruiting on the neck, in most cases at the edge of the leaf sheath at a point where it clasps the neck. The tissue at that point appeared soft and water soaked and of a faint yellowish color. Possibly the outer leaves may have been slightly on the decline, that is, beginning to yellow at the time of inoculation, but this certainly was not apparent. On these plants the fungus clearly showed varying degrees of parasitism, being able to attack the green tissue and also to live on the softened tissue. Certainly, the host tissue was killed in advance of the hyphae. From the behavior of the plants it was plainly evident that most of the infections occurred thru the neck of the bulb and not thru the upper portions of the leaves. The uninoculated checks remained healthy. After these plants were killed by the onion Botrytis and had fallen over to the ground the loose-growing, common Botrytis appeared on some of them as a saprophyte on the dead plants. This experiment was repeated a number of times with similar and convincing results, which proved conclusively that it is comparatively easy to bring about infection of growing onion plants. Apparently the only limiting factor is that the drop of water or suspension does not dry off too quickly.

On April 29, 1915, a deep, greenhouse flat containing 15 onion plants growing vigorously in sterilized soil was placed in the inoculation chamber with a fairly high relative humidity, and atomized with a spore suspension of the onion Botrytis in prune juice. The spore suspension was made by pouring a tube of sterile prune juice upon an agar slant culture which was fruiting profusely and after rubbing off the spores and possibly some pieces of the aerial growth with a sterile platinum needle the liquid was poured into the receptacle of a De Vilbis atomizer. When atomizing the suspension upon the plants some small pieces of the mycelium came up thru the atomizer. Later all of these produced infection wherever they struck. Three days after atomizing, a considerable number of the leaves showed small white spots about 1 to 2 mm. in length (for they had a tendency to run lengthwise of the leaf) scattered over the leaf. By carefully peeling off the outer epidermis of a leaf a typical Botrytis mycelium, 6.6 microns wide, was found penetrating the tissue and growing along the leaf in the direction of its growth. Other typical infections revealed a similar condition. The chlorophyl quickly disappeared from these spots. After remaining in the moist atmosphere of the greenhouse for six days the plants went down rapidly, the fungus fruiting abundantly on the dead and dying, water-soaked-appearing plant parts. After a lapse of one month all of the plants placed in the box were dead, the tops remaining as dried material covered with abundant conidia. It was also evident from this experiment in which sterile prune juice was used instead of sterile water as a medium in which to convey the spores to the plants, that infection takes place readily from the drops of spore suspension.

Ward (1888) in his infection experiments with the conidia of the Botrytis causing a lily disease noted that the young germ tubes, altho unable to infect green leaves and bulbs, made a stronger attack if some saprophytic nourishment was at hand. In order to determine whether there was any difference in the rate of infection from conidia when placed in either sterile water or prune juice and then sprayed on the plants some experiments were made. One of these was as follows: Thirty healthy onion plants about 6 to 8 inches high and growing in a greenhouse bench at the Geneva Station were covered with large glass lantern globes. Ten of these plants were then atomized with the onion Botrytis spores suspended in water, ten with spores in prune juice and ten were retained as checks. All of the glass globes were then closed at the top by wrapping cotton loosely about the projecting leaves and covering the remaining space with a glass plate. Five days later there were unmistakable signs of infection on both the prune-juice-sprayed and the water-sprayed plants, but it could not be seen that the rate of infection was any greater in the case of the prune-juice-sprayed plants except possibly in one or two instances. Typically some of the infections took place on the leaves about the necks and thru those which were very slightly yellowing. At this point it is well to call attention to the work of Brooks (1907, 1908) who found that the conidia of *Botrytis cinerea* were able to infect lettuce leaves which were slightly yellowing, that is, in the incipient stages of decay, while they were unable to attack normal green leaves. This author suggests three explanations for this phenomenon.

In another series of tests comparing prune juice and water as a medium in which to convey the spores to the plants, prune juice gave a slightly greater percentage of infections. Even tho infection of growing plants took place readily when drops of water were present it was evident that the results of these tests were slightly in favor of the prune juice suspension. Apparently, a slight amount of nutrients in the spore suspension has an influence upon the rate of infection.

EFFECT OF SIZE OF DROP OF SUSPENSION UPON INFECTION.

In an attempt to determine the effect of size of drop of spore suspension upon infection of the onion eight healthy plants were selected. Three of these were atomized with spores of the onion Botrytis in water, the atomizer covering the plants with a very fine mist. Three of the plants were sprayed with an onion-Botrytis-spore suspension using a tin atomizer of the hinge type which placed the suspension on the glaucous leaves of the plants in small drops very similar to drops of rain or dew. Two plants were retained as checks. All of the plants were then covered with bell jars. In the course of six days the inoculated plants showed abundant infection on the leaves and stems. The checks remained healthy. While there was not a marked difference shown in amount of infection thru either the small or the large drops, it was clearly demonstrated that the drops of liquid must be of sufficient size to prevent their drying off quickly, a condition such as obtains in the onion fields during a wet season. Observation has shown that following such a season there is considerable neck rot of the bulbs in storage.

INFECTION BY PLACING SPORES BETWEEN LEAF SHEATH AND NECK.

The possibility of infection taking place by dry spores dropping down in the leaf sheath was, in one instance, tested as follows: Eighteen apparently normal onion plants growing in a greenhouse bench were selected; then, with a sterile platinum needle, dry spores from a pure culture of the onion Botrytis were carefully placed down in the leaf sheath, where it clasps the neck, the leaf being gently drawn aside during the operation. Some of the leaves fitted tightly while others did not. Untreated plants were retained as checks. Fifteen days from the date of inoculation seven of the fifteen plants showed infection, that is, the leaf was wilted, drooping, and appeared as if it had been scalded. On eight of the plants no noticeable infection had taken place. Thirty days from the date of inoculation nine of the fifteen plants showed severe injury from the Botrytis infection. The leaves were dead and nearly dried up and the fungus mycelium was found to have passed down into the necks of the bulbs. The checks remained healthy. It was evident from these and similar tests that the dry spores lodging in the axils of leaves or in the leaf sheath very readily found moisture conditions suitable for germination and later caused infection. It seems fair to assume that this may happen when spores of the neck-rot fungus from piles of decaying onion refuse near onion fields are air borne and find lodgment on the plants of the growing crop.

INFECTION THRU INCISIONS.

Some preliminary experiments designed to test the possibility of securing infection by placing the Botrytis spores in incisions in the side of the bulbs and necks showed conclusively that infection takes place very readily thru such wounds when they are covered with wax or some other similar material to prevent a drying out of the exposed tissue. Following the preliminary experiments eighteen onion plants which had formed bulbs about two inches in diameter were selected. After cleaning the neck with a cloth soaked in mercuric chlorid solution and then washing off with sterile water, an incision was made into the neck about one and one-half inches above the surface of the soil. Dry spores from a pure culture of the onion fungus were then placed in the incisions which were immediately covered with grafting wax. Spores were omitted from some of the incisions, these to serve as controls. Seven days after inoculation every inoculated plant, with a single exception, showed that the fungus had made an attack upon the tissue of the neck. All of the control plants remained healthy. The infections showed as a whitening of the tissue around the point of inoculation. In some cases the diseased area extended one-half to three-fourths inch above the wax covering, and on some leaves the whitened or scalded appearance extended up along the leaf, in one case to a distance of three inches above the point of inoculation. In the course of twenty-one days the disease had progressed to the point where nearly every inoculated plant was completely killed. An examination of the leaf tissue about two inches above the point of inoculation showed it to be completely filled with the fungus mycelium, making it impossible to determine whether it was inter- or intracellular. The tissue became soft and of a pale yellowish color above the areas in which the mycelium could be found. Evidently infection takes place readily thru wounds of this type.

INFECTION BY MEANS OF SCLEROTIA.

That infection of bulbs can take place by means of sclerotia was clearly shown by making incisions into the sterilized sides and necks of healthy bulbs and inserting into the wounds sclerotia picked from pure cultures. These were then covered with wax. The results with bulbs so inoculated were positive while the checks which received no sclerotia gave negative results: Evidently, sclerotia also must be looked upon as a source of infectious material.

INFECTION THRU THE FLOWERS.

While working with inoculation experiments upon the leaves, necks and bulbs of the onion in the Geneva greenhouse a number of seed bulbs or " mother bulbs " placed in a bench produced seed heads. It was noticed that as soon as the flower heads opened, some of them were attacked by the onion Botrytis which was fruiting upon onion stems in the same house. The young germ tubes readily attacked the stigmas, anthers and petioles of the flowers producing a blasting of the flower heads very similar to that which occurred in the onion seed fields.

In experiments designed to test the pathogenicity of the onion Botrytis for the flowers, dry spores placed in freshly opened flowers germinated, and readily attacked and destroyed the non-cuticularized floral parts. The fungus then worked down thru the receptacle and invaded the seed stalk. In the greenhouse, at least, the initial infection of the flowers took place by means of wind-borne spores from diseased plants in adjoining benches. It seems fair to assume that infection of flowers in the onion seed fields could occur in a similar manner. Field observations and all indications confirm this idea.

MYCELIUM IN NECKS OF INFECTED BULBS.

At the conclusion of the greenhouse experiments in 1915 there was a considerable quantity of onion bulbs at hand some of which had matured. Some of these bulbs had shown infection from the neck-rot fungus early in the season, but after the neck and some of the dry, outer skins had been removed the specimens appeared sound even tho somewhat undersized. A careful examination was made of a considerable number of these bulbs in search of the mycelium in the necks. In nearly every instance the mycelium was found between the outer three or four fleshy scales at the necks of the bulbs. In the case of one apparently healthy bulb a mat of mycelium was found between the second and third fleshy scales where it had caused no discoloration of the tissue. Isolations from this mycelium gave a prompt and vigorous growth. When such bulbs were placed in a moist chamber the neck rot developed rapidly.

INOCULATION EXPERIMENTS WITH OTHER PLANTS.

Species of Lilium, including *L. tigrinum*, *L. candidum*, and *L. grandiflorum* were dusted with dry spores and sprayed with various spore suspensions of the onion Botrytis on leaves, buds, and open flowers. These gave negative results in every instance, indicating that under the varying conditions under which the tests were conducted the onion Botrytis is not pathogenic for these species of Lilium.

Greenhouse flats of lettuce plants were placed in an inoculation chamber, at a number of different times, and sprayed with spores of the onion Botrytis suspended in sterile distilled water. In none of the trials did infection from the onion Botrytis follow the inoculations.

Garden peas were started in greenhouse flats filled with sterile sand from a cutting bench. These peas were started for the purpose of testing germination and were planted very close together. When the plants were about five inches high the flats were placed in a moist chamber and atomized with a spore suspension of the onion Botrytis. No indications of infection were apparent until six days later when it was observed that infection had taken place only on the yellowing leaves and stems. After infection had taken place the infected parts became flaccid and water soaked. The fungus then progressed to other parts of the plants killing the tissue in advance of its growth. Attention has already been called to the observations of Brooks (1908) who noted a similar instance in the case of *Botrytis cinerea* on lettuce plants.

SUMMARY AND CONCLUSIONS.

The results of inoculations of dry onion bulbs, and of growing plants, by dusting with dry spores, were negative. However, positive results were obtained from inoculations made in the following ways: (1) By spraying spores on the growing plants, and on the bulbs; (2) by placing dry spores between leaf sheaths and the neck; (3) by placing spores in incisions in the necks and sides of the bulbs; (4) by placing sclerotia in incisions in the necks and sides of bulbs; and (5) by dusting dry spores on the freshly opened flowers. Evidence was secured that drops of liquid in which the spores were suspended must be of sufficient size to prevent their drying off quickly. Some evidence was also brought forth which indicated that a small amount of nutrients in the drop of spore suspension slightly favors infection. An examination of bulbs from plants used in the infection experiments showed the mycelium of the fungus to be present in the necks.

A summary of the inoculation experiments brings forth but one conclusion, viz.: That spores in large drops of water (or sufficient moisture) on leaves, in wounds, and chiefly on the necks of onions (and only onions) produce the typical neck-rot disease. Sclerotia must be looked upon as a source of infectious material. The leaves attached to and clasping the lower part of the neck are very susceptible to attack, especially when they are on the decline, i. e., slightly yellowing.

With the results of the inoculation experiments in mind it was possible to go into the onion fields and storage houses and explain most of the conditions found there. It is believed that in the field the greater part of the infections above ground take place thru the lower leaves and the neck and then work down into the bulbs, the mycelium going into the storage houses in the necks of the bulbs, where it develops rapidly under favorable conditions.

THE PARASITE.

MORPHOLOGY.

Mycelium.— The mycelium is large and coarse, septate, and varies considerably in diameter in the same hypha. In diseased onion bulbs the mycelium frequently occurs as only 4.5 microns wide, while in diseased seed stalks from the field it is often 9 microns wide, the average width being approximately 6.5 microns. It has no regular method of branching, and is not only intercellular but intracellular, it being impossible in many cases to determine this point because of the disintegrated condition of the host tissue (Plate XX, fig. 11). In the bulbs and necks of the host plant the young mycelium is practically colorless. With age the walls become of a sepia tint bordering on a light brown color. Various types of cross connections may be found in the host tissue (Plate XX, fig. 12). The mycelium when crowded down between the bulb scales forms a dirty-white mat. Here, in one instance, it gave an average width of 6.3 microns for a large number of measurements. The mycelium is filled with a dense, fine-grained or minutely vacuolate protoplasm, frequently appearing granular. When grown in solid media weak in nutrients, and in synthetic solutions, the mycelium shows considerable variation in form and method of growth. Fig. 14 on Plate XXI, shows a type of mycelium common in solid media. A type of heavy mycelium is frequently found in pure cultures (Plate XXI, fig. 26). Sporulating filaments not unlike those shown by Beauverie and Guilliermond (1903) and giving the same staining reactions to haematoxylin and methylene blue were found (Plate XXII, figs. 33, 34). The mycelium showed " corpuscular bodies " similar to those shown by these investigators (Plate XXII).

The short, erect conidiophores arise directly from the mycelium either singly or in clusters (Plate XX, fig. 6). They turn from hyalin to light brown and later to brownish black. Upon the host plant the conidiophores are usually unbranched (Plate XX, figs. 6, 9), while in cultures there appears to be a tendency toward branching (Plate XX, fig. 8). The compact clusters of conidia are borne upon vesicles which have a tendency toward a spiral arrangement (Plate XX, fig. 7), and usually upon the upper portion of the conidiophore (Plate XX, fig. 10).

Appressoria.— While studying the behavior of the fungus as it worked its way down the neck of inoculated onion plants it was noted that appressoria or organs of attachment were formed rather profusely. These appeared to have an important relation to the discoloration and solution of the cell walls of the host. The writer's attention was first attracted to these bodies while examining an onion leaf which had been sprayed with a spore suspension of the onion Botrytis. The leaf appeared green and perfectly normal with the exception of small white spots where infection had taken place, and one particular area where the fungus was fruiting abundantly. Around this latter spot the leaf tissue for a distance of about 5 mm. appeared as if it had been scalded. By making thin sections thru the leaf, at a point where the diseased and healthy areas adjoined, and then unrolling the sections, the inner side of the leaf tissue could be examined. This examination revealed a profusely branched mycelium growing upon the inner epidermis of the leaf and extending along in the same direction as the long diameter of the cells, i. e., in the direction of growth. A considerable number of the tips of the hyphae showed knobs or enlargements of various shapes and sizes, many of them being simply enlargements of the tips of the hyphae (Plate XXI, figs. 15–22). In most cases these bodies were full of dense, bright protoplasm, while the mycelium just back of them was vacuolate, and markedly so. The tips of these bodies were surrounded by a film of material which was very refractive to light and which could be rather easily demonstrated by staining in weak gentian violet for a few seconds. This condition as described was found to be very common in the inoculated plants and those showing natural infection from the field. Quite frequently the mycelium thickens slightly just back of the appressorium. The young mycelial elements may also become markedly flattened where they come in contact with the culture dish (Plate XXI, fig. 32). The bodies appear simply to apply themselves to the host tissue following which there is a softening of the tissue and it takes on a water-soaked appearance.

The chlorophyl disappears and the mycelium branches and penetrates the tissue at will. It was found, however, that similar, but more complex and larger organs of attachment were formed when the fungus was grown upon culture media. Here they may be formed as single flattened hyphal tips where they come in contact with the glass side of the culture dish. (Plate XXI, fig. 30), or as a repeatedly branched tassel-like cluster of hyphae with their tips flattened against the glass walls (Plate XXI, figs. 25, 27, 31). In many instances two or three hold-fasts could be seen close together, apparently due to the branching of the mycelium just above (Plate XXI, figs. 28, 29). In the center of many of the hyphal tips as they come in contact with the glass a bright, clear spot is to be seen, while around the thickened hyphal walls a brownish film of a mucilaginous nature is visible. Ward (1888) and Kean (1890) studying a lily disease caused by a Botrytis observed a " glairy film " around the tips of the short hyphae forming the organs of attachment and concluded that this was a ferment excreted by the tips of the hyphae. They point out that these are the bodies described by De Bary (1884) and called by him " Haft-organen."

Aderhold (1896) mentions the fact that the appressoria of Venturia are frequently surrounded by a zone of mucilaginous material. Voges (1910) also noted in Venturia a " gelatinous envelope " around the appressoria of that fungus. He suggests that these are functional in attachment of the fungus. Smith (1900), Price (1911) and Cook and Schwarze (1913) figure organs of attachment of *Botrytis* spp., not greatly unlike those of the onion Botrytis.

Blackman and Welsford (1916) have demonstrated that the germ tubes of *Botrytis cinerea*, when they have reached sufficient length (about one spore-diameter), " possess a mucilaginous investment " by means of which the germ tube is aided in exerting sufficient pressure to penetrate the leaf tissue. These authors further state that this method of penetration is impossible in the absence of the mucilaginous investment of the germ tube, but that " penetration can occur without the development of an appressorium."

Sclerotia.— The typical, firm, black sclerotia may be found upon any or all parts of the onion bulbs in storage. These vary in size from 1 mm. in width to large crusts covering nearly the entire side of a bulb (Plate VII). The sclerotia vary in shape from small roundish bodies 1 to 2 mm. wide and flattened on one side to large, contoured and folded crusts covering the diseased bulbs. Frequently there are indications of striations on the sclerotia. These run in the same direction as the veins of the bulb scales. It is not uncommon to find the sclerotia massed together in the form of a sclerotial crust encircling the neck of the bulb. Sclerotia were found which presented the appearance of an inseparable layer of dull black enamel over the onion scales. Typically, the sclerotia have a tendency to round off on the upper surface while the lower side is flattened or even slightly hollowed out and inseparable from the onion bulb scales or tissue. When found between the bulb scales they often take the shape of the cavity which they fill. The sclerotia are of the cartilaginous type, having a hard outer cortical covering or layer filled with a white mass of mycelium closely pressed together forming a pseudoparenchyma. Cultural experiments, to be discussed later, show that the presence of a certain amount of moisture is favorable to the development of sclerotia. Sclerotia are not formed readily in cultures except where certain conditions prevail. They are formed where the mycelium is pressed together at the point of contact of fungus and glass, very often appearing as centrifugally formed crusts. It was observed that in many cases the appressoria are incipient stages of the sclerotia (Plate XXI, figs. 23, 24). At first the sclerotia are dark flesh color rapidly becoming black.

The sclerotia germinate by the ends of hyphae pushing out from the pseudoparenchyma and forming tufts of conidiophores bearing conidia similar in shape, size, color, and germination to those found on the mycelium (Plate XX, fig. 13). All attempts to induce the sclerotia to germinate in any other manner than by pushing out typical conidiophores which bear conidia or by the formation of a mycelium were failures with the exception of one remarkable instance which will be described in a later paragraph. No apothecia were formed on any of the sclerotia. Sclerotia taken from onions in various storage houses and from pure cultures were sterilized in several different ways, then placed in test tubes of sterile soil, in deep and shallow dishes of sterile sand and loamy soil, and kept in the laboratory, out-of-doors, and in the ice box for varying lengths of time; but in all cases the tufts of conidiophores were produced when germination took place. Sterilized sclerotia which were thrust into sterilized cornmeal moistened with water, produced, in germination, an abundant mycelium which gave a fairly profuse crop of sclerotia thruout the medium. In other experiments sterilized sclerotia were floated upon sterile water and prune juice. Some of the sclerotia sank to the bottom of the liquid; but many of those which remained at the surface produced conidiophores and conidia. When the sterilized sclerotia were thrust into prune, potato, and onion agar in petri dishes, pure cultures of the fungus were invariably obtained.

A number of sclerotia were separated into two lots before sterilizing — one lot comprising the small sclerotia, the other the larger ones. When placed on moist sterile sand to germinate both sizes responded with the usual crop of conidiophores and conidia. However, it was noticed that the small sclerotia were slightly more viable than the larger specimens.

Sclerotia subjected to the stimulating action of drying, and to fumes of chemicals such as ether, chloroform and alcohol gave results no different from those previously noted.

From the germination tests made upon hundreds of sclerotia the following points were secured: The most rapid and the highest percentage of germination occurred when the sclerotia were kept out-of-doors or in an ice box. A fairly low temperature (about 10° C.) greatly stimulates germination. Drying and exposure to sunlight reduced the viability of the sclerotia. If the sclerotia placed on sterile, moist sand or similar media did not germinate, or even after they had produced a crop of conidiophores, they readily broke down leaving the outer cortical layer in the form of a crust.

The length of time during which the sclerotia remain alive is very important. A fairly large percentage of the sclerotia were found to be dead at the end of one year. One lot of sclerotia kept for two years in the laboratory showed a very weak germination in a very few instances at the end of that time. Results of the tests with sclerotia indicate that under average conditions the life of the sclerotium in moist soil is but a few months, possibly only a few weeks.

It was noted above that in one particular instance germination took place other than by the production of the typical tufts of conidiophores or the formation of mycelium. This was the case with a lot of sclerotia picked from onion bulbs in storage early in 1913. These were sterilized by being soaked in sterile distilled water, then immersed in a 0.1 per ct. mercuric chlorid solution for two minutes, and again washed in sterile distilled water. The sclerotia were then picked from the water with sterile forceps and placed upon moist sterile sand in petri dishes. Some of the dishes were kept in the laboratory in a dark closet, the others were placed in an ice box. On June 6, 1913, it was noted that two of the plates in the ice box showed that germination had taken place by the production of tufts of conidiophores bearing conidia, while upon a third plate there were no typical conidiophores but instead there were formed stalk-like bodies with masses of conidia at their tips. (Fig. 1, plate XXVIII). The stalks of these peculiar structures were black while the tips were white. These bodies were 20 mm. high and 3 mm. wide. Cultures made from the knobs of these structures upon onion agar gave pure cultures of the Botrytis. The conidia found on these knobs gave an average measurement of 11.3 microns long and 5.7 microns wide. A further analysis of these structures showed the stalk of one of them to be made up of 16 conidiophores. No further development was noticed in these structures.

Conidia.— The pale colored or hyalin conidia are borne upon stout, short, erect conidiophores, these being very rarely over 1 mm. high, usually much shorter. The conidia are oblong to elliptical in form, often slightly tapering at both ends (Plate XIX, fig. 1). The short tip or sterigma may often be seen at the narrower end of the conidium. The conidia have been found to vary in size from 7.1–16.2 x 3.8–6.3 microns, the average from hundreds being 10.3 microns long and 5.1 microns wide.

The conidia fail to germinate in a saturated atmosphere. In water they are wetted with difficulty, but soon absorb water and germinate rapidly, usually by one, occasionally by two, germ tubes, forming considerable mycelium in 24–36 hours (Plate XIX, fig. 3). The mycelial branches unite very frequently, forming unions (Plate XIX, fig. 4), also the tips of hyphae form cross connections (Plate XIX, fig. 5).

While examining some old cultures of the fungus there were found at the bottom of the slant in the test tube, round, dark brown conidia about 3 microns in diameter and having very thick walls, while at the upper part of the slant typical elliptical conidia were formed. It appeared that conditions of nutrition caused the formation of these peculiar conidia. Price (1911) described some peculiar spore forms of Botrytis. Perhaps these round conidia observed by the writer are similar to the microconidial form of fructification mentioned by Istvanffi (1905), and Brooks (1908) who worked with *Botrytis cinerea.*

Comparative morphology.— The cultural characters of the onion Botrytis were critically compared with those of other forms or species of Botrytis, since during the course of the investigation of the onion Botrytis fungus the writer had opportunity to isolate a considerable number of forms or possibly species of Botrytis from a number of hosts. Some of these were living saprophytically on dead plant parts, while others were active parasites. At the outset, the possibility of the onion Botrytis being the common gray mold found upon greenhouse plants presented itself. As soon as the onion Botrytis was placed in pure culture and compared with the so-called *Botrytis cinerea* Pers., it was plainly evident that the organisms compared were entirely distinct species. The onion Botrytis possessed a low, dense, felt-like aerial mycelium bearing elliptic, smoke-gray conidia on very short conidiophores, while *B. cinerea* showed the loose, cottony, cinereous mycelium with long erect conidiophores, bearing the pale, globose conidia (Plate XIX, figs. 1, 2). The writer has isolated the loose-growing common Botrytis from dead onion leaves and from the tips of growing onion leaves where dying back had taken place due to the disease called " white tip." It was also isolated once from onion bulbs stored in a storage house along with a quantity of carrots which were severely attacked by *Sclerotinia libertiana* and overgrown with Botrytis. In another instance both the common Botrytis and the onion Botrytis were isolated from the perennial or tree onion growing in a private garden. Here, the onion Botrytis was acting as a destructive parasite killing the lower leaves and necks of the clusters of green bunching onions, while the common Botrytis was living as a saprophyte on the dead onion leaves. In Plate XXVII the onion Botrytis is shown as culture 12091 with its low form of growth, and the common Botrytis as culture 12091–1 with its loose, cottony mycelium completely filling the culture tube. Both cultures are of the same age and on the same kind of medium. Plate XXVII also shows four cultures of Botrytis picked at random from stock cultures. The cultures serve to show the dense, felt-like growth of the onion Botrytis as compared with the loose, cottony growth in the three other cultures, and also of that of a large number of isolations of Botrytis from a number of greenhouse plants. The writer will not venture a statement as to the classification of species of Botrytis showing the loose, cottony growth. While greatly resembling each other, it may be said that they present some distinctive cultural characteristics. The onion Botrytis has been carefully compared with forms of Botrytis from every available source among which were *B. parasitica* said to be the cause of the " Dutch bulb disease," and *B. peoniae* the cause of the Botrytis disease of peonies. None of these seems at all similar to the onion Botrytis. To summarize, the writer is confident that the onion Botrytis is responsible for the neck-rot disease of onions and is a separate species. Smith (1900, p. 397) appears to be the first in America to point out that the Botrytis causing the onion disease " was not a typical cinerea " because of its " low, dense manner of growth." He gives some excellent illustrations of the conidia and conidiophores of the onion Botrytis comparing them with *Botrytis cinerea.*

The name of the fungus.— Considerable confusion has existed as to a name for this fungus since it has apparently been known under a number of different names. Sorauer (1876) appears to be the first to record a disease of onions which may be the neck-rot disease. In 1886 the same author described a disease of onions due to a Botrytis which evidently included a sclerotial stage. He referred the Botrytis stage to *Botrytis cana* (Pers.) Fr., and the sclerotial stage to *Sclerotium cepae.* Again, in 1908, Sorauer discussed a Botrytis disease of onions and referred the Botrytis stage to *Botrytis cana* Kze. et Schm., and the sclerotial stage to *Sclerotium cepivorum* Berk.

Frank (1880, 1896) reported a Botrytis disease of onions. He reiterated Sorauer's statements as to the possible identity of the Botrytis stage of the fungus with *Botrytis cana* Pers., and the sclerotial stage to *Sclerotium cepae* Berk. et Br. This author (1896) stated that he noted a Botrytis on *Allium ursinum* near Leipzig which was similar to that observed by Schröter.

Halsted (1890), in what appears to be the first report in America of an onion bulb disease due to a Botrytis, states that *Botrytis parasitica* Cav. may be the cause.

In 1894, Massee, writing in the *Gardeners' Chronicle,* described an onion disease due to a Botrytis with an ascogenous stage. This disease, he states, was due to *Sclerotinia bulborum* Wakker. He figures a Peziza form not unlike *Sclerotinia libertiana* Fckl., and a Botrytis form with globose conidia very similar to *Botrytis cinerea* Pers. Oudemans (1884) and others state that no Botrytis stage has been found for *Sclerotinia bulborum* (Wakk.) Rehm., which was described by Wakker (1883) as causing a disease of hyacinth and other bulbs, and that none occurs. At any rate the description and figures presented by Massee cannot apply to the fungus causing the neck rot of onions. Again, in 1903 and 1914, Massee restated that a destructive rot of onions due to *Sclerotinia bulborum* occurs. Until the connection between the Botrytis and the *Sclerotium* forms has been demonstrated these descriptions only lead to endless confusion.

In 1900 Smith pointed out that the onion bulb rot due to a Botrytis and observed by him near Munich was not typical *B. cinerea* because of its low, dense manner of growth and smaller spores. This author also doubted the possibility of the onion rot being due to *Sclerotinia bulborum* Wakker as suggested by Massee.

Voglino (1903) made a careful study of a disease of *Allium sativum* which occurred in Italy and was able to prove conclusively that the onion disease caused by a Botrytis as described by Sorauer (1886) and Prillieux (1897) is not due to *Sclerotium cepivorum* Berk., but he suggested that it may be a Botrytis described by Schröter on *Allium ursinum* and one found by himself as occurring on onions and referred to *Sclerotium ambiguum* Duby. This author stated that due to the kindness of Professor P. A. Saccardo he was able to examine in the Exsiccati of Libert[1] typical examples of *Sclerotium cepivorum* Berk. (*S. cepae* Berk. et Br.) and to determine the identity of these with those found on *Allium sativum*. This author further stated that the sclerotial form of Libert and of the specimens which develop in various parts of Italy are constantly spherical in form as is true of the form found by Garavaglio in rice plants and described by Cattaneo[2] under the name *Sclerotium oryzae*. These correspond to the description by Berkeley as reported by Fries[3] and by Saccardo[4] for *Sclerotium cepivorum* Berk. (*S. cepae*

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[1] Cryptog. exs. Ard. n. 238.

[2] Saccardo, Syll. Fung. 14: 1153.

[3] Summa Vegetabilia Scandinaviae, page 478.

[4] Syll. Fung. 14: 1151.

Berk. et. Br.). "Minutum, sphaeroideum, gregarium, atrum, tegumento distincte parenchymatico fuligineo, intus cellulis varie ramosis intricatis albis farctum." Voglino carefully worked out the conidial form of this fungus which was very abundant on the bulbils and which he described as *Sphacelia allii* distinguishing it by the following characters: "Effusa bulbos, *Allii sativi* crustaceo-pulvinata, roseola, sporophoris e strato proligero oriundis, ramulosis, hyalinis, dense fasiculatis septatis, 40–50 microns longis; conidiis sphaericis, hyalinis, quandoque catenulatis, 1-guttulatis 3–4.5 microns diam." This important work performed by Voglino and to which attention has been called by Delacroix (1909, p. 401) and others, places beyond all doubt the possibility of the neck-rot disease of onions being due to the fungus *Sclerotium cepivorum* Berk.

Clinton (1903–1904) in reporting a Botrytis as causing a serious onion bulb rot of white onions in Connecticut suggested that possibly it is the same as the Botrytis bulb rot of onions and hyacinths in Europe, where it is known in its perfect form as *Sclerotinia bulborum* altho he stated that no perfect or ascospore stage has been noted in Connecticut. He further emphasized the possibility that it is not specifically different from the common Botrytis which is found so commonly on greenhouse plants. According to this author's description of the fungus and its behavior, supplemented by cultures which the writer has secured from diseased onions from Connecticut, no doubt is entertained but that it is identically the same fungus as is found in New York, Michigan, and elsewhere causing the neck rot of onion bulbs.

Bruck (1907) made note of a disease of onions in Europe due to a Botrytis with a sclerotial stage which he suggested is *Sclerotium cepae* Lib. This fungus was found both in the field and in the storage house.

According to Jarvis (1909) a disease of onions in the form of a rotting of the bulbs at the neck before and after storing occurred in Connecticut. This author referred the trouble to the same fungus (a Botrytis) as reported by Clinton (1903) and also recommended a similar method of treatment.

Selby (1910) discussed a "dry or black neck-rot" of onions which occurs in Ohio and causes heavy storage losses when white onions are stored. Altho he does not state that a Botrytis stage occurs he figured a diseased specimen which is typical of the Botrytis neck-rot disease. He stated that the disease is due to *Sclerotium cepivorum* Berk.

In reporting for the state of Oregon, Jackson (1914) mentioned a serious storage rot of onions due to a Botrytis. The disease is known there as onion stem rot and gray mold, and is said to be favored by moist weather conditions, poorly ventilated storage houses, and immaturity of the stock.

According to Hanzawa (1914) a disease of onions due to a Botrytis which has a sclerotial stage occurs in Japan. Hanzawa figured a Botrytis with oval to oblong spores, and stated that they measure 8.4–16.8 microns long and 6.3–10.5 microns wide. The conidiophores are branched at the tips and are about 1 mm. long. He further stated that the fungus resembles *Botrytis cinerea.* The small, round or elliptical sclerotia germinate by forming the Botrytis stage but not the apothecial stage.

In a recent publication Humbert (1916) discussed the same onion disease as reported by Selby for Ohio at an earlier date. He stated that the disease is disseminated by means of spores, but does not intimate that the fungus is a Botrytis. However, he affirms that the causal organism is *Sclerotium cepivorum* Berk.

As far as the writer is able to determine from a thoro search of literature the following fungi from the genus Sclerotium have been reported upon species of Allium. These will be compared with the fungus studied:

1.— *Sclerotium ambiguum* Duby.[1] For this species the sclerotia are described as being minute, ovate or orbicular, with the margins elevated, and having a dark interior. This species has no Botrytis stage and does not resemble the onion neck-rot fungus in the sclerotial stage.

2.— *S. Brassicae* Pers.[2] which is reported as occurring on the scapes of *Allium victorialis* has no Botrytis stage, and has sclerotia which are oblong, flat, 4–8 mm. long, and the interior becoming black. Not at all similar to the onion Botrytis.

3.— *S. cepivorum* Berk. (*S. cepae* Berk. et Br.)[3] A discussion of this fungus has been given in a previous paragraph. Its minute, round sclerotia which have as a conidial stage, a Sphacelia as found by Voglino (1903), exclude it from identity with the neck-rot fungus.

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[1] Duby, Bot. Gall. **2**:875; Saccardo, Syll. Fung. **14**:1150.

[2] Saccardo, Syll. Fung. **14**: 1164; Lindau, Rab. Krypt. Fl. **1**^{9}: 674.

[3] Saccardo, Syll. Fung. **14**: 1151; Lindau, Rab. Krypt. Fl. **1**^{9}: 661.

4.— *S. durum* Pers.[1] has sclerotia which are 4–6 mm. long, sometimes 12 mm. long and as wide, at first under the epidermis then becoming free. This species is reported by Lindau to occur on the stem of *Allium* sp.; also, that *Botrytis cinerea* is said to develop from the sclerotia. It is not similar to the onion Botrytis sclerotia since the latter are in most cases inseparable from the host tissue.

5.— *S. inconspicuum* Lib.[2] which is reported to occur on putrid onion bulbs has very small, brown sclerotia not at all like the sclerotia in question.

6.— *S. pulveraceum* Dur et Mont.[3] has round-globose, small (0.2–0.5 mm.) sclerotia which have been found in the bulbs of *Allium pallens*. These cannot compare with the sclerotia found in the onion bulbs attacked by the neck-rot fungus because of their small size.

7.— *S. tulipae* Lib. var. *Hyacinthi* Guep.[4] which is reported as occurring on the stems of *Allium vineale* has thin, ovate-oblong, minute sclerotia from which *Botrytis parasitica* Cav. is said to have been produced. Their occurrence on the stem, and their small size and thinness distinguish them from the sclerotia of the onion Botrytis.

Six species of Botrytis have been reported as occurring upon some parts of plants of *Allium* sp. These, also, will be critically compared with the onion Botrytis studied.

1.— *B. aclada* Fres.[5] is reported as occurring on the dying scapes of Allium. However, it does not correspond to the onion Botrytis because it has ovoid-oblong conidia which are said to be one-half the size of those of *B. acinorum* Pers.; that is, 5–7 x 3.5–4.5 microns.

2.— *B. cana* Kze. et Schm.[6] is reported to have large oval spores. According to Ward (1888, p. 360) *Botrytis* (*Polyactis*) *cana* Berk. (*B. cana* Schmidt) has conidia measuring 30–33 x 15–8 microns. The conidia of neither of these species compares at all with the long elliptical conidia of the onion Botrytis.

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[1] Saccardo, Syll. Fung. 14: 1165; Lindau, Rab. Krypt. Fl. 1^9: 674.
[2] Saccardo, Syll. Fung. 14: 1151; Lindau, Rab. Krypt. Fl. 1^9: 662.
[3] Saccardo, Syll. Fung. 14: 1151.
[4] Saccardo, Syll. Fung. 14: 1172; Lindau, Rab. Krypt. Fl. 1^9: 677.
[5] Saccardo, Syll. Fung. 4: 131; Lindau, Rab. Krypt. Fl. 1^8: 285.
[6] *Myk. Hefte* 1: 83; Saccardo, Syll. Fung. 4: 129.

3.— *B. cinerea* Pers.[1] has been reported on *Allium* sp. The writer has found this species growing upon dead parts of onion plants in a number of instances; but its roundish, oval conidia are not to be confused with the long elliptical conidia of the onion Botrytis responsible for the onion neck-rot disease.

4.— *B. fulva* Link[2] which is reported as occurring upon the dead stems of *Allium cepa* has spherical, yellow-brown conidia measuring 4–4.5 microns in diameter and having fine spines.

5.— *B. parasitica* Cav.[3] which is reported by Höhnel as occurring on *Allium ursinum* has conidia which are ovate and large, measuring 16–20 x 10–13 microns, or nearly twice the dimensions of the onion Botrytis conidia.

6.— *B. vulgaris* Fr. var. *interrupta* Fr.[4] has been reported as occurring on scapes of Allium. This species has globular-elliptical conidia slightly longer than those of the onion Botrytis. However, it has typical constricted or interrupted conidia, which definitely excludes it from being the onion neck-rot fungus.

From the foregoing statements it will be seen that the fungus causing the neck-rot disease of the onion has been incorrectly assigned under a number of different names none of which is available for it. Accordingly, the name *Botrytis allii* n. sp., is proposed with the following description:

Colonies dirty white shortly becoming smoke gray (Ridgway, 1912, pl. 46), old cultures approaching the drab of Ridgway, close, dense, feltlike; mycelium septate, coarse, with no definite method of branching, 4–9 microns in diameter, filled with fine-grained or minutely vacuolate protoplasm, giving rise to short erect conidiophores; conidiophores numerous, septate, erect, usually about 0.5 mm., rarely over 1 mm. high, occurring either singly or in clusters, not often branched on the host plant, but with a tendency toward branching in cultures, branches short and indicating a spiral arrangement, conidia-bearing on upper portion ($\frac{1}{4}$–$\frac{1}{3}$), conidial clusters approximate, not widely separated; conidia hyalin or very dilutely colored, elliptical, often slightly tapering at both ends, 7.1–16.2 x 3.8–6.3 microns, average 10.3 x 5.1 microns, attached by a short sterigma to the vesicles in small clusters; sclerotia dull-black, firm, white inside with a dark outer cortical layer which is usually very finely wrinkled or striated, flattened or concave on the side by which they are inseparably attached to the host tissue, strongly convex above, single sclerotia 1–5 mm. in diameter usually several to many united in clusters or large sclerotial crusts which may be several centimeters in diameter. Sclerotia germinate by hyphae which immediately produce conidiophores. No apothecia produced.

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[1] Saccardo, Syll. Fung. 4:129; Lindau, Rab. Krypt. Fl. 1^8:284.

[2] Saccardo, Syll. Fung. 4:123; Lindau, Rab. Krypt. Fl. 1^8:280.

[3] Saccardo, Syll. Fung. 10:536; Lindau, Rab. Krypt. Fl. 1^8:292.

[4] Fr. Syst. Mycol. 3:398; Saccardo, Syll. Fung. 4:129.

Hab. Parasitic on leaves, flowers, and bulbs of *Allium cepa*.

PHYSIOLOGY.

Sources of pure cultures.— The pure cultures used in these studies were obtained from a number of different sources, including isolations from onion bulbs from Bermuda, Connecticut, Massachusetts and Texas. Isolations were made from Michigan material in the form of flower heads, bulbs in the field and storage, over-wintered necks in field, and perennial onions. A large number were made in New York from sclerotia, from bulbs in field and storage, from over-wintered onion refuse in the field, from flowers and stalks from the seed crop, and from the necks of plants in the growing crop. Whenever possible the diseased plant part was placed in a damp chamber where a profuse production of conidia would take place. The conidia were easily picked off by touching the tufts of conidiophores with a drop of sterile water on a platinum needle and then transferring to onion agar. When diseased onion bulbs were used they were either split open with a sterile knife, or the outer scales peeled off and placed under a bell-jar in a fairly damp atmosphere. Here, abundant fruiting took place on the newly exposed surface and isolations were made as noted above.

Growth on vegetable tissue.— Of the various raw plant parts used for media, including sour and sweet apple, carrot, tomato, potato, pea stems, string beans and sterile onion bulbs, the last were by far the most satisfactory, giving the most abundant growth. The raw onion scales were prepared by carefully peeling off the dry outer scales, then immersing the bulb in a 0.1 per ct. solution of mercuric chlorid for two minutes to disinfect the surface. The outermost scale was cut off with a flamed knife. The remaining scales were then sliced off and dropped into sterile 100 cc. Erlenmeyer flasks. Very often it was difficult to get the scales free from micro-organisms. After standing three days to test sterility the flasks were inoculated by dropping spores in upon the moist scales. Such cultures gave a prompt growth which was in full fruit in eight days, the scales being completely hidden under a dense, close-growing mycelium and multitudes of smoke-gray conidia. Where the flasks were loosely plugged and the media dried out readily, no sclerotia were formed, but a heavy crop of conidia was produced. All of the other raw plant tissues were prepared in essentially the same manner.

Conidia, and even bits of mycelium, when placed on slices of sterile raw carrot in a sterile moist chamber gave a very slow, scanty growth on moist pieces, but no growth where the tissues were not moist or had dried out.

It was noted that the growth taking place on cooked vegetables was usually many times greater than that upon raw vegetables. This was true of all the various kinds tested with the possible exception of the onion bulbs. On string beans immersed in mercuric chlorid, then washed in sterile water and clipped into short pieces, placed in small flasks and inoculated with spores, the first growth appeared on the cut surfaces then slowly developed over the uncut surfaces. On this raw vegetable the growth was very scanty and produced no conidia. On string beans placed in test tubes and autoclaved at 110° C. for twenty minutes the growth was very profuse. A heavy crop of conidia was produced but no sclerotia were formed.

Cultures on pea stems, sterilized and raw, gave a very striking contrast. On the raw stems the Botrytis developed very scantily, while on stems sterilized in the autoclave for twenty minutes an abundant crop of conidia was produced. The color of the growth in these flasks was drab (Ridgway). Numerous organs of attachment were formed, but no typical sclerotia.

On sterile raw lettuce leaves in deep culture dishes no mycelial development took place except where abundant moisture was present in the form of drops. On lettuce leaves autoclaved in 250 cc. flasks a dense growth occurred. The conidia were typically smoke gray and very abundant. In the autoclaved lettuce flasks it was seen that the liquid below the surface growth turned black while the liquid in the uninoculated checks remained clear. This suggested the possibility of a biochemical change taking place in the inoculated flasks following the development of the fungus.

Pieces of the raw flesh of the apple, when taken under aseptic conditions and placed in flasks and compared with similar pieces in flasks sterilized in the autoclave at 115° C. for fifteen minutes, gave a marked contrast. On the cooked apple the growth developed rapidly producing a few conidia and numerous appressoria at the edges of the cultures where the growth came in contact with the flask. On the raw apple the growth was very slow and but few conidia were formed. The fungus changed the color of the apple tissue from a light flesh color to a brown. The aerial mycelium was dirty white color in raw apple cultures.

Raw, sweet apple flesh, aseptically prepared, was compared with similarly prepared raw, sour apple as a medium in flask cultures. The mycelial growth at first was slightly more abundant on the sweet apple, following which a more abundant conidial production took place. On the sour apple flesh very few conidia were produced but a considerable number of sclerotia 1 to 3 mm. in diameter were formed at the bottom of the flask where the pieces of apple were in contact with the glass and also at the edges of the pieces of apple. This apparent difference may have been due to a variation in the moisture content of the two varieties of apples, the sweet apples being more pulpy. The relation of the reaction of the medium to the development of the fungus is discussed elsewhere in this paper.

On blocks of raw potato tuber taken aseptically, but very slight growth took place, while on blocks of potato tuber autoclaved for fifteen minutes an abundant development of dense, close-growing, felt-like mycelium occurred. In the autoclaved potato flasks hundreds of small typical sclerotia varying in size from 1 to over 2 mm. in diameter were produced over the upper surface of the medium. Along the sides of the flasks, at least, all the intermediate stages between the holdfasts and the fully developed sclerotia could be seen.

The sclerotia which are formed at the bottom of the cultures are usually irregular in shape, appearing as sclerotial crusts and often filling and taking on the shape of the space between the pieces of potato tuber. Frequently one finds a row of typical sclerotia along the edge of a potato block where it touches the side of the flask, indicating that the mycelial strands when compressed or forced tightly together readily form sclerotia.

A quantity of small green-onion bulbs was sterilized in mercuric chlorid, then washed in sterile distilled water and placed in 500 cc. flasks. When these bulbs were inoculated with dry spores of the onion Botrytis the young germ tubes attacked the tissue of the bulbs reducing them to a soft brown mass upon which a profuse crop of conidia and sclerotia developed.

Growth on plant broths, and on solid nutrient substances with and without broths.— Broths from various plant parts were used alone and added to various solid nutrient substances. This series included: lima-bean broth, potato broth, apple broth, prune juice, plum juice; cornmeal alone and saturated with prune juice, apple and onion broths; and sterile bread crumbs saturated with prune juice, apple, potato and onion broths. There was little noticeable difference in the rate and manner of growth of the fungus on any of these media. The sterile cornmeal plus prune juice gave the best development of sclerotia, while the cornmeal plus potato broth presented the most unfavorable conditions for sclerotium formation. The bread crumbs plus onion broth gave the best development of sclerotia together with the most profuse crop of conidia. Organs of attachment were also abundant on this medium. It is worthy of note that the color of the growth on onion broth was similar to the typical smoke gray which characterizes the growth on the host. On all of these media, wherever the mycelium came in contact with the side of the culture flask black masses of organs of attachment were formed. When sufficient moisture was present sclerotia were formed wherever crowding of the mycelium took place. Many of the sclerotia germinated almost immediately after formation by sending out tufts of conidiophores which later bore conidia.

Growth on synthetic solutions.— An attempt was made to grow the fungus on Knopp's and Uschinsky's standard synthetic solutions and also upon Coons' synthetic solution (Coons, 1916, p. 752). Upon these solutions only a slight amount of conidium formation resulted. The growths obtained are of interest, however, since they showed development of abnormal forms of conidia, some of which were extremely long and misshapen.

Growth on various agars.— In addition to plain, nutrient-glucose, and starch agar, other agars were made by adding agar flour to various plant extracts. Those worthy of mention are apple, potato, prune, lettuce-leaf (with and without peptone), and onion agar.

The growth obtained on the plain, nutrient-glucose and starch agars were very scanty. Only a few conidia and organs of attachment, and no sclerotia developed. Of the other kinds of agar tried, by far the most typical and abundant conidial development took place upon the onion agar with tomato agar coming next in order. The growth on potato and prune agar was rather slow, and with very scant conidium formation on the latter.

Since the onion agar [1] gave the most typical development of the fungus and because of its ease of preparation it was used almost exclusively for carrying stock cultures and for growing crops of conidia and sclerotia for inoculation experiments.

While the results with these media are by no means complete, they indicate that moisture is a factor which very largely determines the formation or non-formation of sclerotia and also that abundant sclerotial formation and conidial production may not occur on the same culture.

A critical study of over 1000 cultures of the fungus shows that it possesses constant and easily recognized cultural characteristics on several media. Upon onion agar, which is typical of the host plant, the most distinctive and reliable cultural characteristic of *Botrytis allii* is its low, dense, felt-like, close-growing, dirty white aerial mycelium, which gives rise to short conidiophores bearing the eliptical smoke-gray (Ridgway, 1912) conidia. With age, the surface growth may become light drab (Ridgway) and even approach the drab of Ridgway. This characteristic persists for generations.

Thermal relations.— Determinations for the thermal death point (T. D. P.) were made with spores of the fungus, using the capillary tube method as described by Novy (1899). Thin, straight capillary tubes of even caliber were prepared, by drawing out to approximately 24 inches pieces of glass tubing about five inches long; these when carefully drawn provided four tubes. The usual method for determining the T. D. P. of fungus spores is to use tubes of culture medium which are immediately poured into petri dishes as soon as time and temperature exposure are secured. The spores of Botrytis become wet with difficulty, but it was found that by pouring 10 cc. of sterile distilled water on an agar slant culture of the fungus which was fruiting freely, the spores were washed off readily and could be drawn up into the capillary tubes by touching the surface of the liquid upon which the spores were held in the form of a film. After being filled and sealed the capillary tubes were placed upon a rack made of wire netting which was then supported in a water bath kept at correct temperature by the aid of a thermometer suspended in the liquid. At the end of the time allotted for the various temperatures, a tube was taken from the rack, wiped dry, one end scratched with a file then snapped off and flamed. By holding the flamed open end in the end of an agar slant tube of onion agar while the closed end was passed thru a gas flame the contents of the capillary tube were ejected upon the agar slant surface. Spores germinate in about 36 hours and growth can be detected macroscopically. The results are shown in Table I.

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[1] The onion agar was made up by slicing 200 grams of clean sound onion bulbs into somewhat less than 500 cc. of water, cooking for a few minutes and steaming in the autoclave for 30 minutes at 110° C. The onion juice was then strained thru cotton and filter paper and added to 15 grams of agar flour dissolved in 500 cc. of water. The volume was then made up to 1000 cc. with water, filtered, tubed, and autoclaved for 15 minutes at 115° C. This medium gives a very slight acid reaction, frequently being but slightly above the phenolpthalein neutral point.

TABLE I.— THE EFFECT OF TIME AND TEMPERATURE UPON SPORES OF THE ONION BOTRYTIS.

Temperature.	TIME IN MINUTES.					
	1.	2.5.	5.	7.5.	10.	
Deg. C.						
35	+	+	+	+	+	T. D. P.= 50°C. for 10 minutes. + = growth in tube. — = no growth.
40	+	+	+	+	+	
45	+	+	+	+	+	
50	+	+	+	—	—	
55	+	—	—	—	—	
60	—	—	—	—	—	
65	—	—	—	—	—	
70	—	—	—	—	—	

In order to compare the test-tube-media method with the capillary-tube method as described and used in the trials reported above, onion gelatin in tubes of equal diameter and thickness of wall was used. After inoculation with the Botrytis spores the gelatin was carefully brought to the melting point, then placed in the water bath along with control tubes and one tube of gelatin with a thermometer immersed in it. After the temperature had reached the desired point in the control tubes the tubes were exposed for ten minutes with but very slight variation of temperature when the plates were poured and cooled immediately. The results of these tests, which compare closely with those of the capillary-tube method, are as follows:

At 37° C. for 10 minutes — Copious germination on the plates.
“ 40° C. “ “ “ — Fair germination on plates.
“ 45° C. “ “ “ — A few colonies.
“ 50° C. “ “ “ — Three colonies.
“ 52.5° C. for 10 minutes — One colony.
“ 55° C. for 10 minutes — No growth.
“ 60° C. “ “ “ — No growth.

It was found by similar tests with pure cultures which had not sporulated that the thermal death point of the mycelium was approximately the same as for the spores. O'Brien (1902) found that the mycelium of *Botrytis vulgaris* was killed at 50° C. for exposures of 30 and 60 minutes.

Some attempts were made to determine the ability of the fungus to withstand low temperatures by the following method: Tubes of sterile distilled water were poured upon agar slant cultures well covered with spores, then poured back into the original tubes thus giving dense spore suspensions. Other agar slant cultures showing a profuse growth of spores, and some showing no spores but copious mycelial growth were placed, along with the water-spore suspensions mentioned above, in a receptacle, then carefully surrounded with cracked ice and salt. At the outset, the thermometer, also packed in the ice and salt mixture, registered below 0° C., and the water was frozen solid. These tubes remained in the ice box for three days when cultures were made from them. Transfers from all of the tubes gave positive results. In this connection it is also interesting to note that Brooks and Cooley (1917) found that *Botrytis cinerea* when inoculated into apples grew at 0° C. This fungus also grew in cornmeal agar in petri dishes at 0° C.; and the spores germinated in cornmeal agar at 0° C. in one month. According to Hoffman (1860) the spores of *Botrytis vulgaris* germinated very near the freezing point and did not lose their vitality at temperatures below freezing; however, such temperatures were fatal to germinating spores.

The results of the study of the temperature limits for the life and growth of *Botrytis allii* on culture media show that: (1) The high thermal death point of 50° C. (122° F.) will permit this fungus to survive in the field in any of the onion growing states; (2) the cultures, spores, or mycelium are not killed by freezing — or low temperatures. It is, therefore, reasonable to conclude that the fungus may readily live over from year to year in the onion fields.

Relation to environmental factors.— Reaction to temperature:— The fungus evidently passes the period of dormancy in the soil at relatively low temperatures; therefore, the ability of the fungus to withstand cold is important. Trials bearing upon the reaction of the fungus to temperature were conducted as follows: Immediately after inoculation with the Botrytis spores, tubes of onion agar were placed in each of nine compartments of a home-made differential thermostat designed to give a wide range of temperatures. A compartment at one end of the apparatus contained about one liter of water which was renewed automatically and which was kept at the boiling point by an electric heater. At the other end of the row of compartments a larger space was filled with ice and salt. When kept carefully iced the variation in the range of temperature of each compartment was insignificant. The results of a single trial are as follows:

TABLE II.— GROWTH OF BOTRYTIS AT DIFFERENT TEMPERATURES ON ONION AGAR.

Compartment.	Temperature.	Growth at end of 10 days.
	Deg. C.	
1	8	Small amount of growth; no spore production.
2	12	Fair amount of growth; few spores produced.
3	20	Growth abundant; much fruiting.
4	24	Growth abundant; medium amount of fruiting.
5	26	Growth abundant; a few spores produced.
6	28	Growth medium; very few spores produced.
7	31	Growth slight; no spores produced.
8	40	Growth faint.
9	45	No growth perceptible.

From the results of these tests it is evident that a temperature of about 35° C. is unfavorable for the growth of the fungus even tho, as reported in a previous paragraph, the fungus may survive a temperature of 50° C. A slight amount of mycelial growth with no spore production took place at 8° C. The optimum temperature was quite clearly shown to be approximately 20° C. That spore production took place only between the temperatures of 12° and 28° C. is rather interesting and significant in view of the recent work of Beauverie and Guilliermond (1903), who worked on the polymorphism of Botrytis. By controlling certain factors, mainly moisture and temperature, they produced a strain of Botrytis which did not form conidia. Coons (1916), who reviews the literature bearing upon the subject in a recent paper, has shown conclusively that the temperature limits for reproduction of the fungus pathogene studied by him are much narrower than those for growth.

Reaction to light:— Of all of the various tests made with cultures of the fungus upon various kinds of media and placed in light and darkness, with the same degree of aeration, no indications were noted that there was any marked response to either of these factors. Darkness appeared to favor slightly the formation of sclerotia. It was only when the fungus was grown upon a medium poor in nutrients that a slight response to light in the direction of increased production of conidia could be detected. This latter observation appears to be in accord with the results of Lendner (1896) who tested the effect of light upon Botrytis among other species of fungi. Reidemeister (1909) reports that with *Botrytis cinerea*, light favors the production of conidia while darkness favors the formation of sclerotia.

Reaction to aeration.— Of all the factors tested, the reaction of the fungus to aeration was most marked, it being sensitive to the oxygen supply. Cultures of the fungus in sterile flasks of liquid media, vegetable tissues, and bread soaked with various fruit juices, which were sealed by covering the cotton plug with paraffin, showed only a very scanty production of conidia in most cases, while unsealed flasks produced an abundant crop. The growth, which was very abundant in the sealed flasks, was mainly in the form of a close-growing, dense mat of white mycelium and a fairly abundant crop of sclerotia. The effect of aeration upon the formation of sclerotia and organs of attachment or appressoria was well illustrated in a series of tests in which tubes of onion agar inoculated with the onion Botrytis spores were poured in petri dishes which were then placed in deep battery jars kept moist by means of wet cotton. While no conidia were produced on these cultures, a very profuse formation of organs of attachment and many sclerotia resulted. Rings of small sclerotia were formed in the petri dishes where the medium was thin due to the intentional tipping of some of them while they were cooling (Plate XXIX, fig. 1). All of these observations appear to agree with the conclusions of Reidemeister (1909) who investigated the conditions of sclerotium and sclerotial ring formation of *Botrytis cinerea* and demonstrated that increased transpiration favored sclerotium formation and retarded production of conidia.

All evidence pointed to the fact that under increased transpiration conidia were formed abundantly, but no sclerotia; while under conditions of very meager transpiration a generous crop of sclerotia, many appressoria, and no conidia were produced. With these facts in mind it was possible to explain the conditions found in the storage houses, where, in a very humid atmosphere the fungus developed mainly sclerotia, while a profuse formation of conidia resulted when the diseased bulbs were removed to the outside air or to the drier atmosphere of the laboratory. Large quantities of diseased bulbs, partially or completely covered with sclerotia, when removed from the storage houses to the dump heap were soon covered with a dense crop of conidia. These serve as a virile source of infection to the growing crop of onions.

Reaction to desiccation.— The length of time that the conidia may live was deemed very important. From dried cultures in test tubes on onion agar the conidia gave a rapid response to conditions favoring germination after 21 and 24 months. At the end of three years the conidia from these dried up cultures showed a very weak germination, and a very low percentage. The length of life of the mycelium in dried cultures was by no means as long as that of the conidia, it being approximately nine months to a year. The longevity of the sclerotia when dried was approximately double that of sclerotia kept in moist earth.

Reaction to moisture.— The fact that the diseased onion bulbs, when in a very damp storage house, showed a copious sclerotium production with but scanty conidium formation suggested moisture as an important factor. When bulbs bearing these sclerotia were transferred to another storage house with a drier atmosphere, other factors being approximately equal, a copious conidium production took place. These indications were substantiated by the results of moisture tests with culture media in tubes and flasks in which excessive moisture was shown to delay conidium production somewhat and to facilitate sclerotium formation. To a series of 200 cc. flasks each containing 10 grams of sterile cornmeal there were added 10, 20, 30, 40, and 50 cc. of sterile water. These were inoculated with the onion Botrytis spores. In these flasks, as they stood, drying took place and brought about a range of conditions. The best condition for the production of conidia appeared to exist in the flask containing 20 cc. of water, while conidia matured slightly earlier in the flask containing 10 cc. of water. The most favorable conditions for sclerotium formation existed somewhere in the range of flasks containing between 20 cc. and 30 cc. of water and the 10 grams of cornmeal. Evidently, the range of moisture conditions which favor the formation of conidia and sclerotia is not a wide one.

Biochemical relations.— Relation to acids and alkalis.— A study of the reaction of some of the common media used in determining the cultural characters of this fungus indicated that the range of growth and production of conidia was a rather wide one. In order to determine the optimum acidity or alkalinity which favors growth and spore production the fungus was grown in flasks of prune and onion juice which were first neutralized and then reinforced with definite amounts of malic acid or sodium hydroxid. Definite amounts of the sterilized and neutralized plant juices were placed in flasks to which were added the required amounts of sterilized acid or alkali. The flasks were then inoculated with spores. Table III gives the results of a single test with prune juice. The titrations are expressed in terms of Fuller's scale, computed in terms of cubic centimeters of normal hydrochloric acid or potassium hydroxid in a liter by titrating 5 cc. with N/20 standards, using phenolpthalein as an indicator.

From these results it will be seen that this fungus produced a crop of conidia on some of the alkali series, the growth in these flasks having the typical smoke-gray color. On the acid series the growth was considerably lighter in color. The vegetative growth in the neutral flask was whiter in color than that in any of the other flasks. The rate of growth was very materially retarded in the concentrations greater than —20° and +20°. While the range over which conidium production may take place is comparatively wide, the range of vegetative growth is surprisingly wide, being approximately —100° to +100° Fuller's scale. The results seem to indicate that the strongest mycelial growth is associated with low acidity or alkalinity, while a comparatively high concentration of either favors conidium production. It seems apparent, however, that the solution used (prune juice) is rather rich in nutrients, especially sugars.

TABLE III.—THE EFFECT OF ACIDITY AND ALKALINITY OF THE MEDIUM UPON GROWTH AND CONIDIUM FORMATION OF *Botrytis Allii*.

Reaction, Fuller's scale.	GROWTH DURING EXPERIMENT: TIME, 1 MONTH.	
	Mycelium.	Conidia.
—300	None	
—200	None	
—100	Very little	
— 50	Small amount	Fruiting abundantly.
— 30	Small amount	Fruiting abundantly.
— 20	Medium amount; felt thin	Abundant conidia.
— 10	Fair growth	Fruiting moderately.
— 5	Good growth	Very few conidia.
0	Best felt of mycelium	Fruiting less than —30.
+ 5	Slightly better than —5	Fruiting much less than on alkali series.
+ 10	Slightly better than —10	Fruiting much less than on alkali series.
+ 20	Slightly better than —20	Fruiting much less than on alkali series.
+ 30	Medium amount	Fruiting much less than on alkali series.
+ 50	A little	Fruiting much less than on alkali series.
+100	Very little growth	Very few conidia.
+200	Scant	Very few conidia.
+300	None	

That this species of Botrytis is not especially sensitive to acids is in accord with the results obtained by Ward, who states (1888, p. 334) that with the lily Botrytis the degree of acidity tolerated may be considerable. On the other hand, Zschokke (1897) stated that *Botrytis cinerea* shuns sour fruits such as currants and sour apples. Under the heading of different media tested the writer gives some results from the use of sweet and sour apples. The acid and alkali tolerance of this fungus is remarkable. It must be a factor in the growth of an organism living on a highly nitrogenous substratum, where ammonia accumulates.

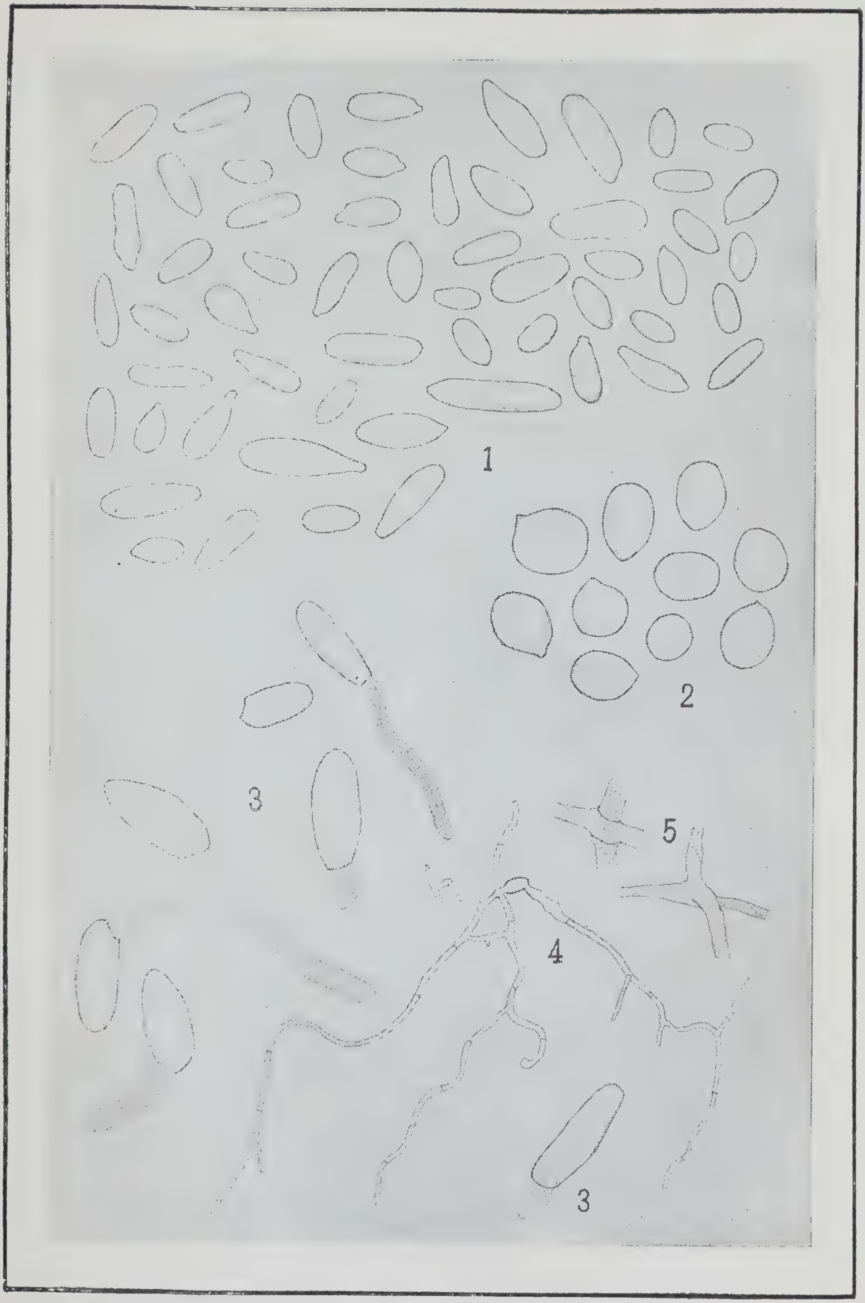

Plate XIX.
(See back of Plate XXIX.)

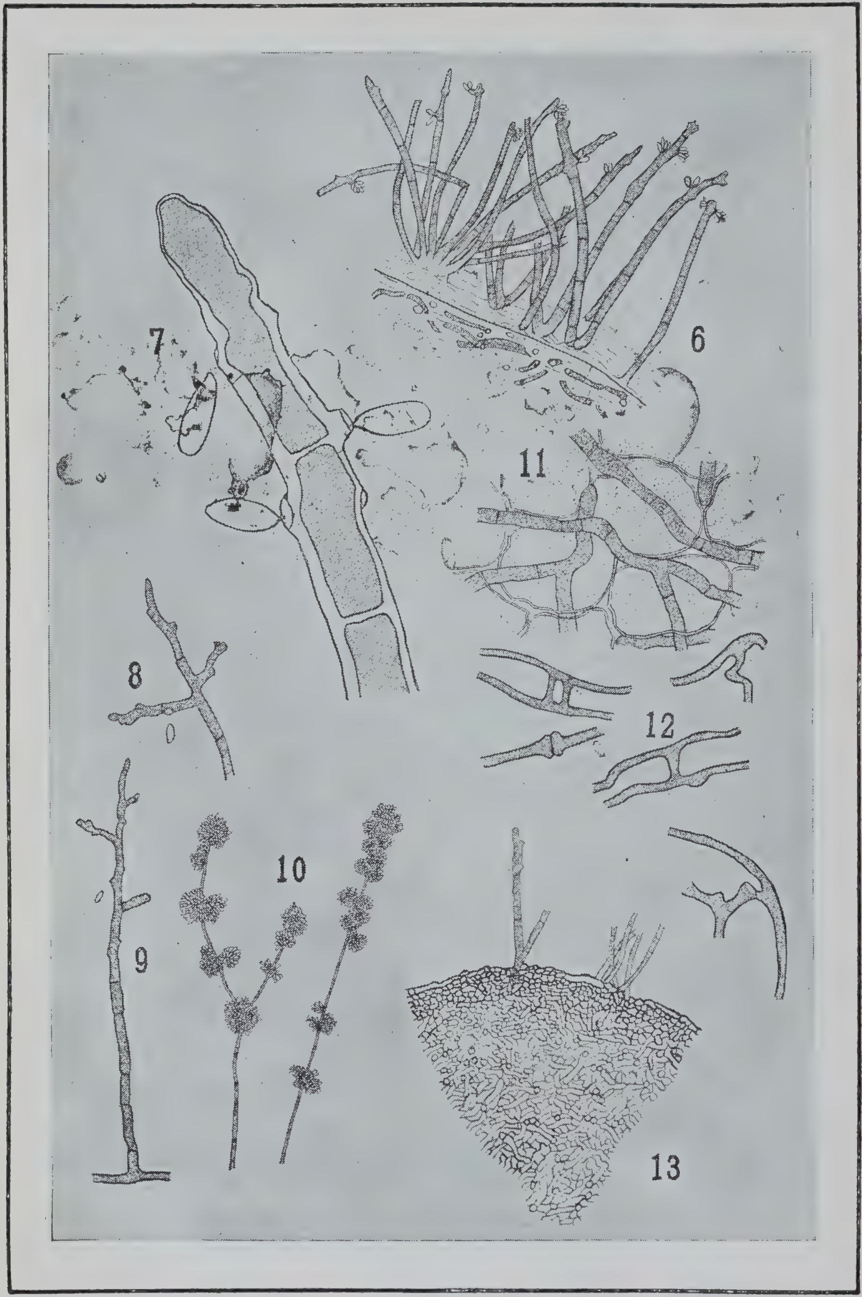

Plate XX.
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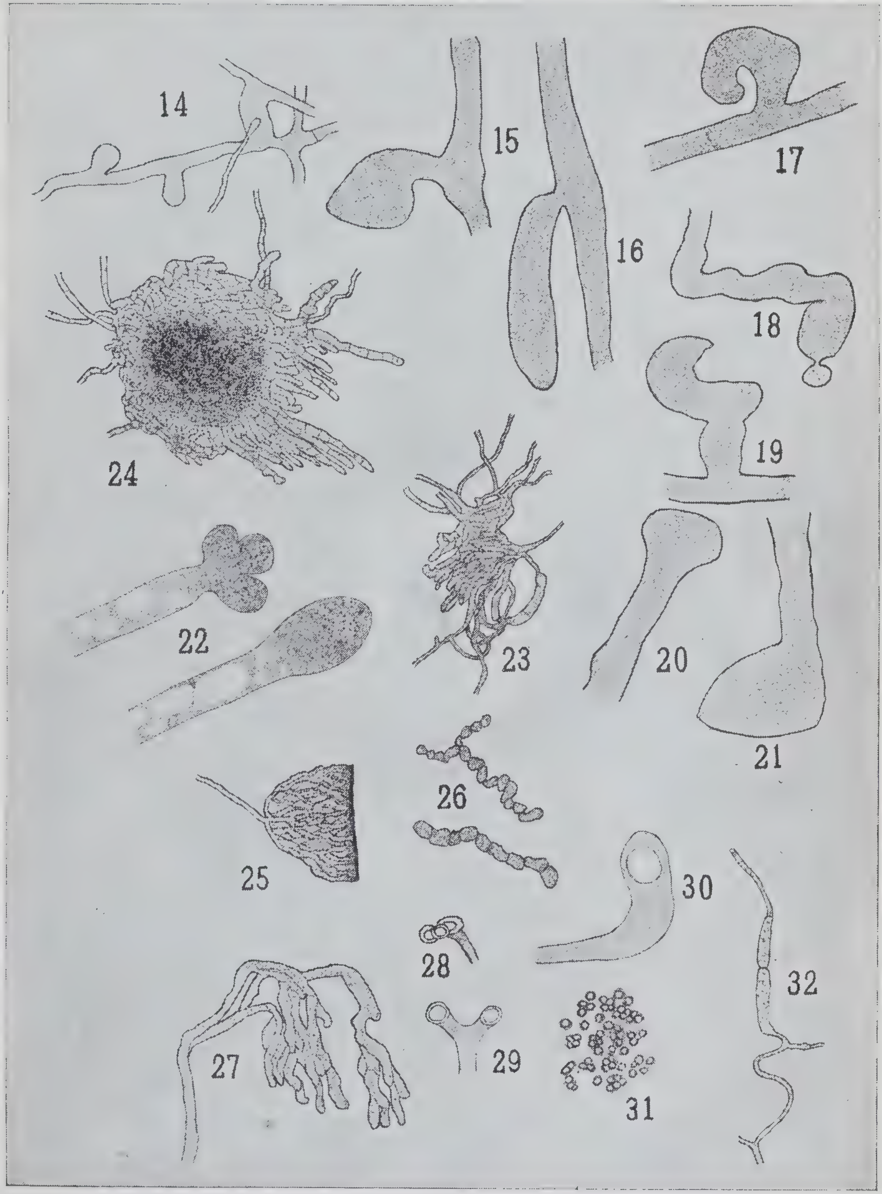

Plate XXI.
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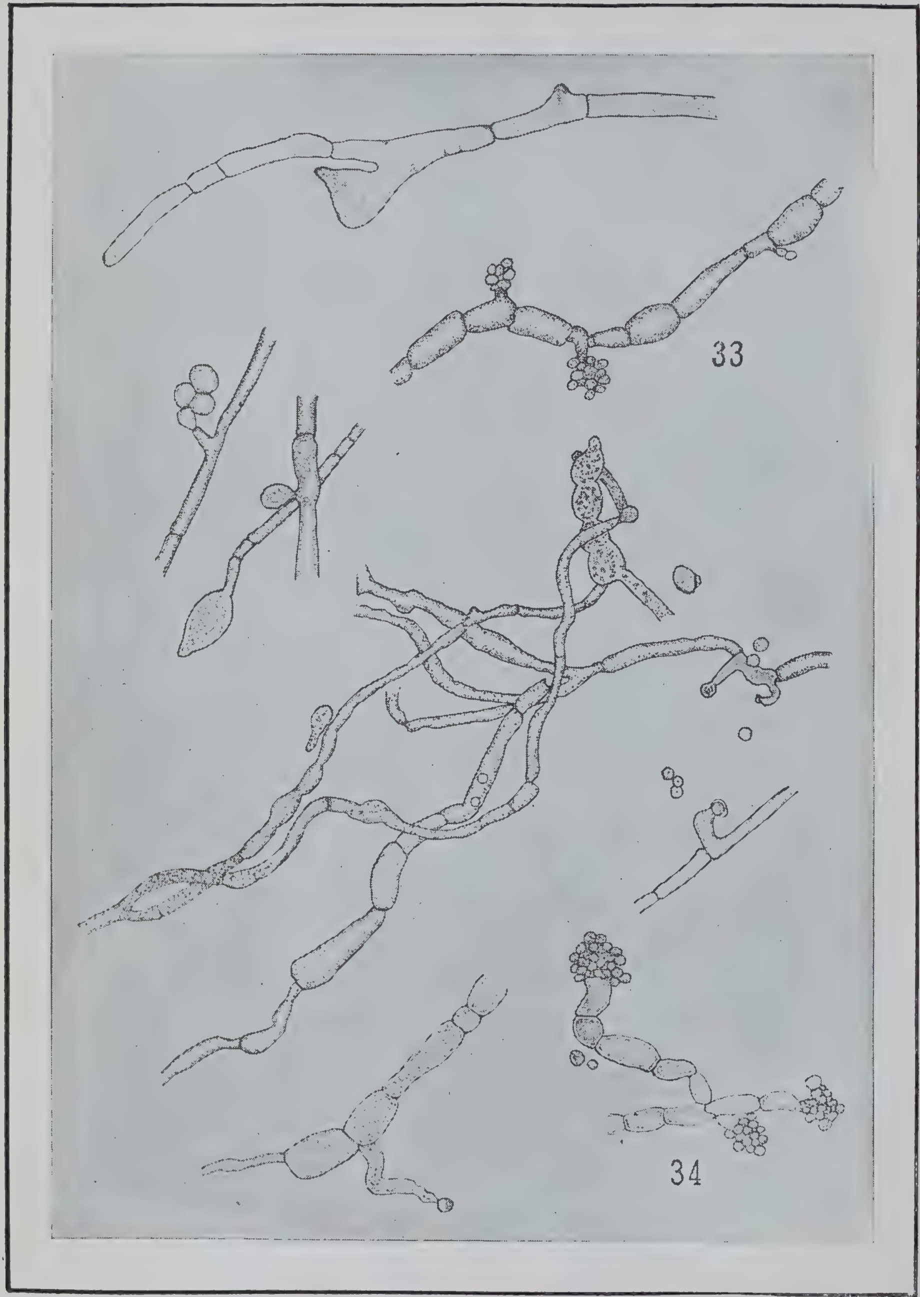

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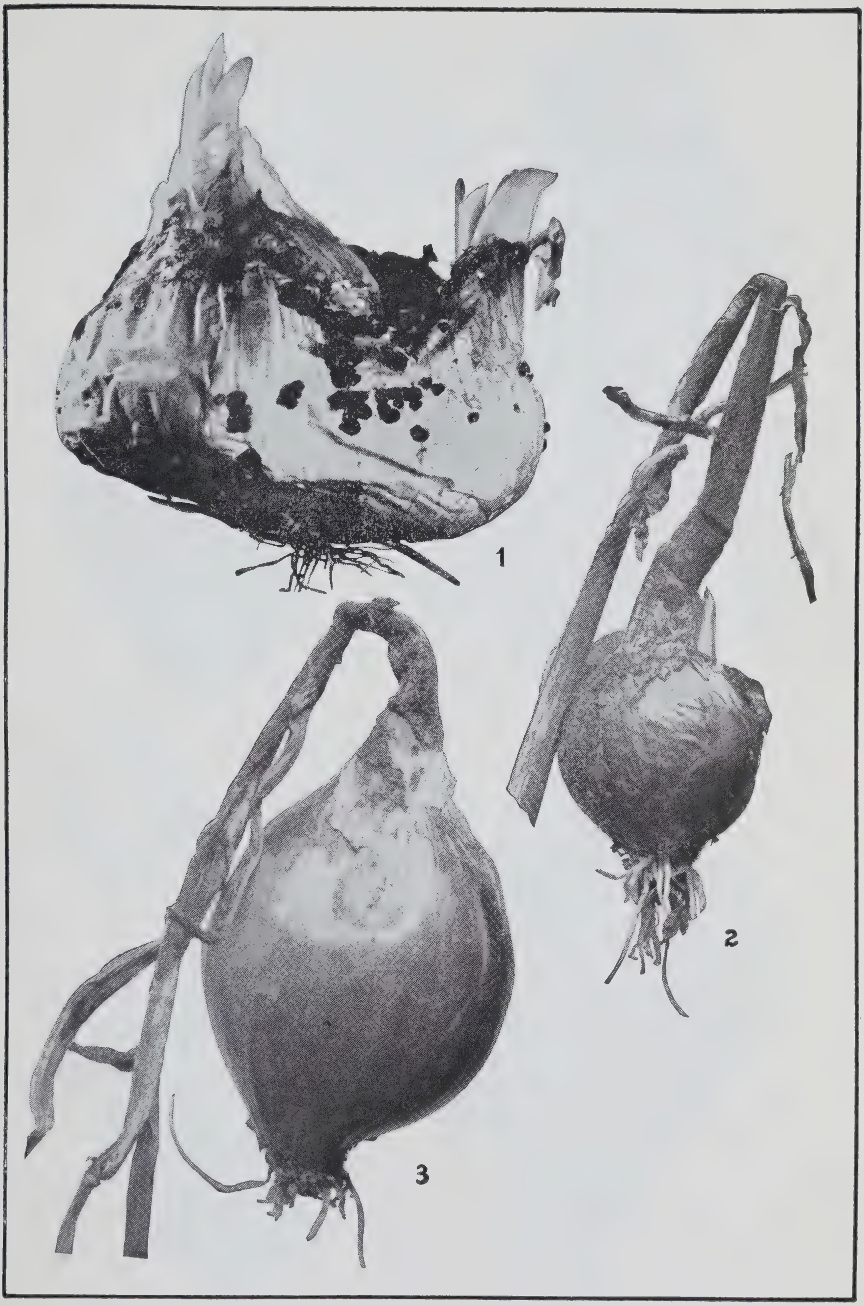

Plate XXIII.
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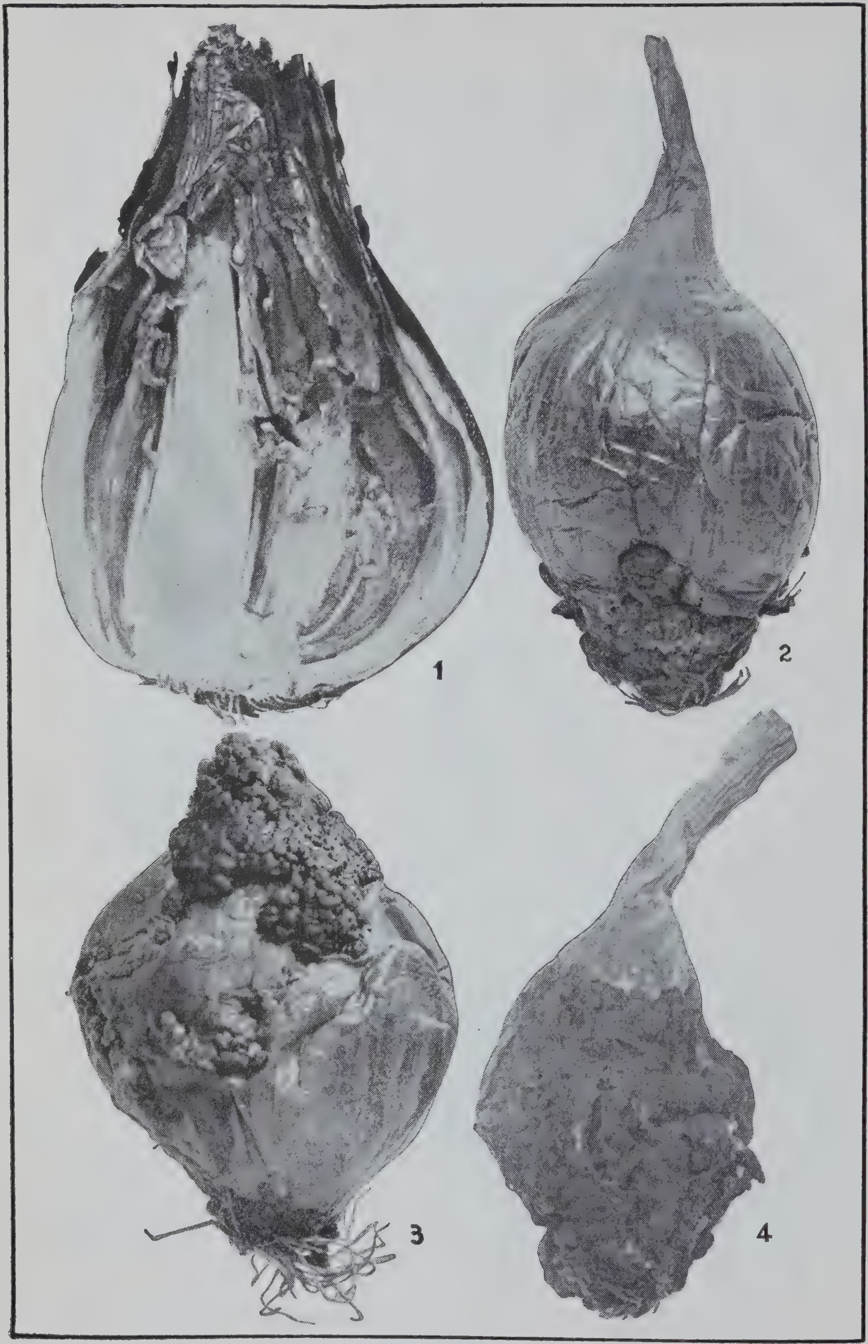

PLATE XXIV.
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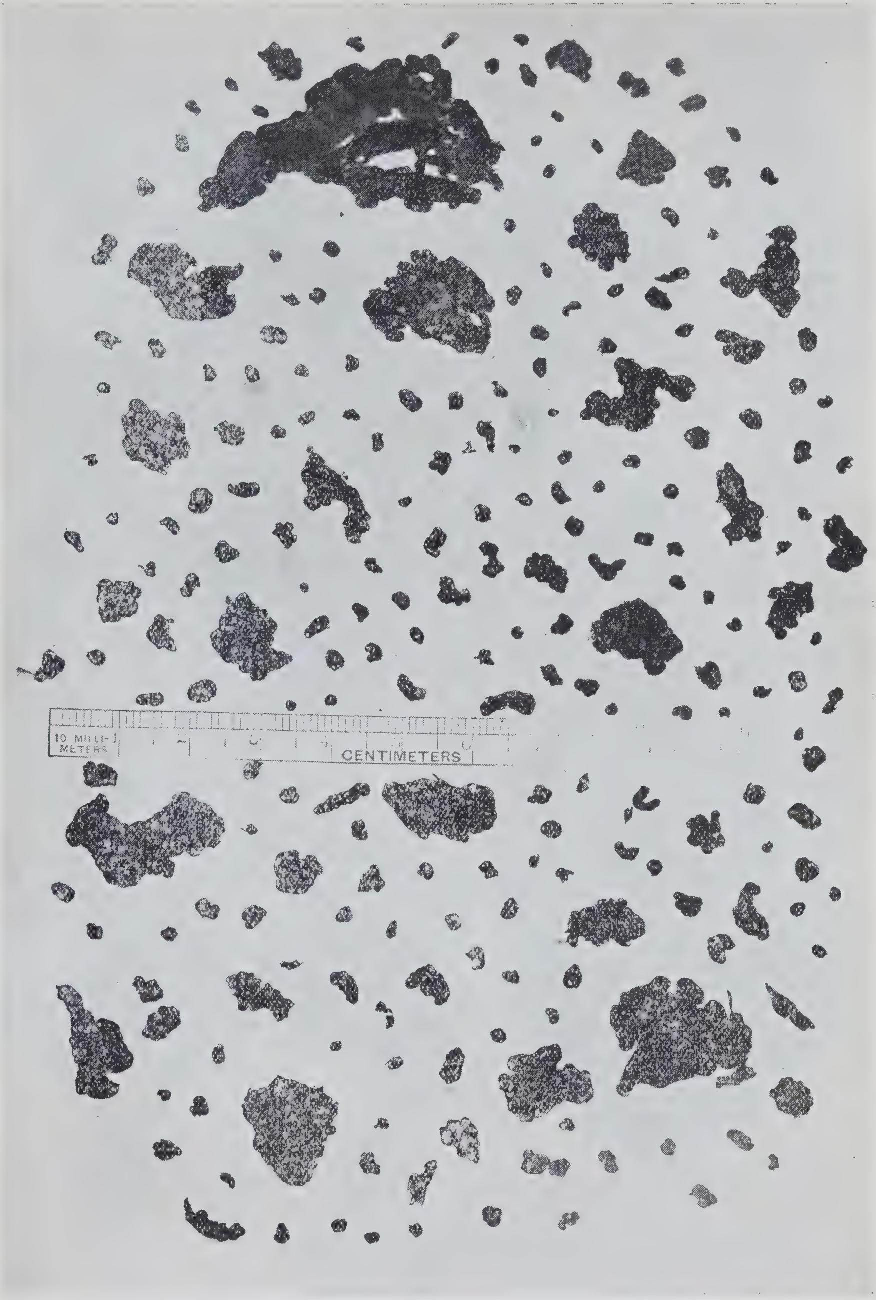

PLATE XXV.
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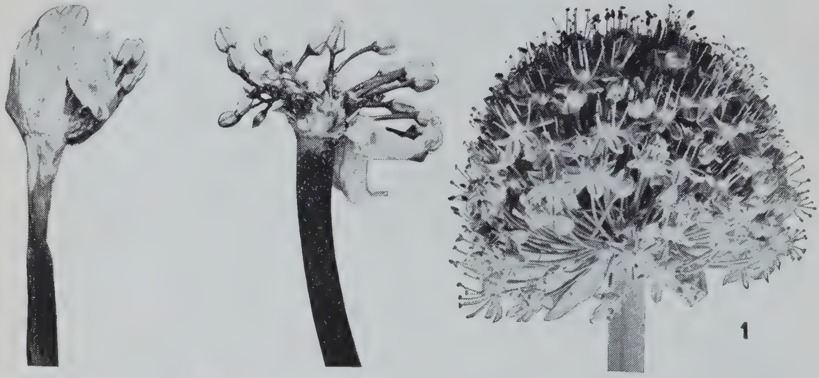

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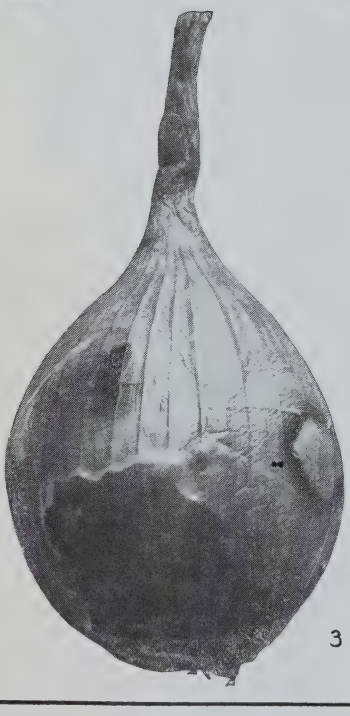

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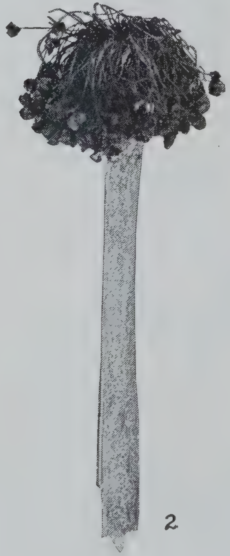

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Plate XXVI.
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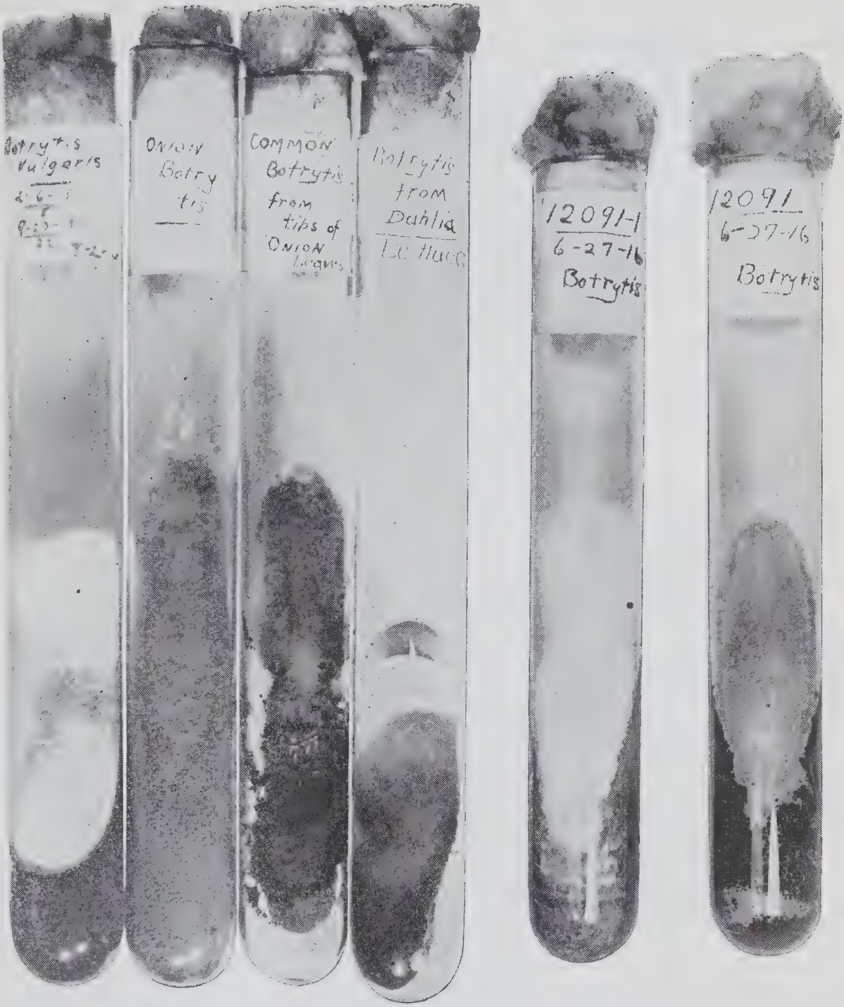

Plate XXVII.
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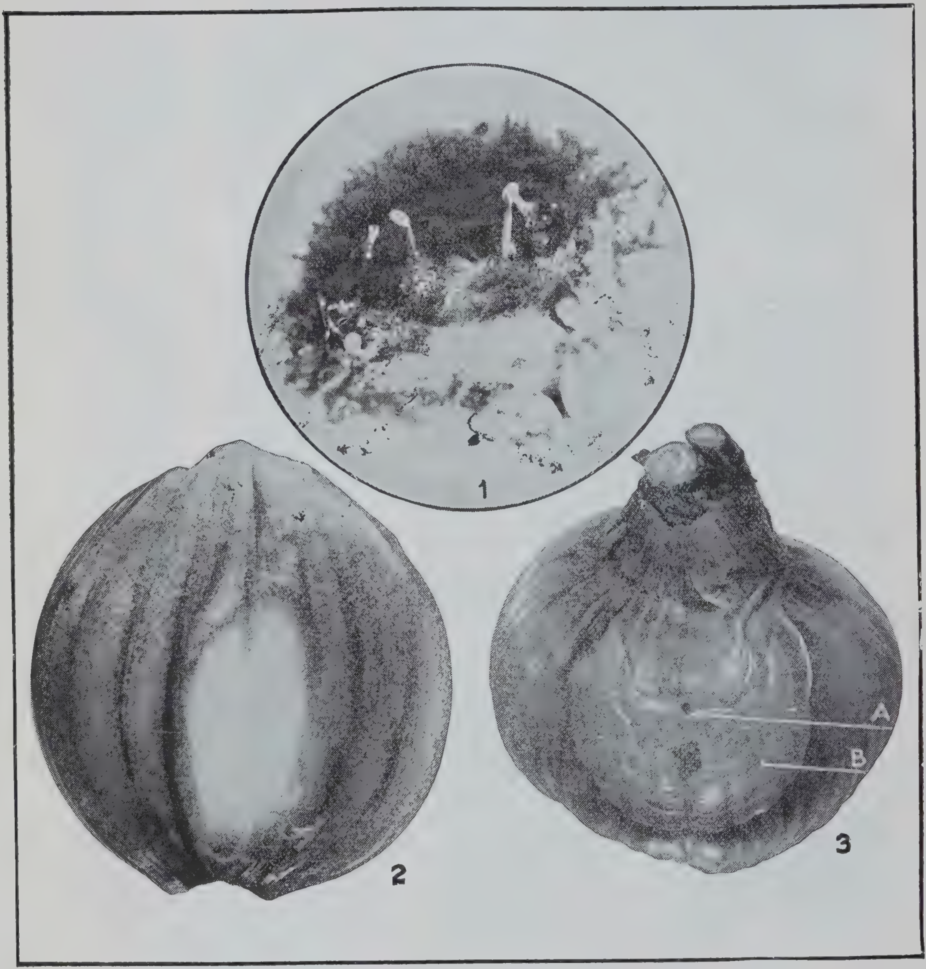

PLATE XXVIII.
(See back of Plate XXIX.)

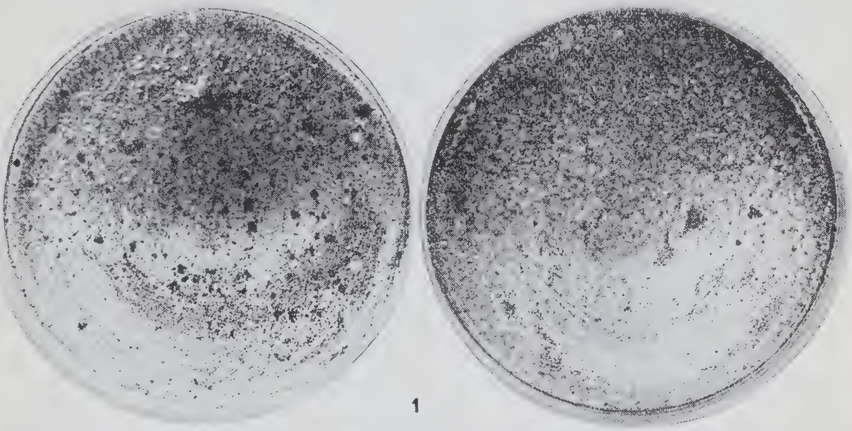

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Plate XXIX.
(See back of Plate.)

EXPLANATION OF PLATES.

PLATE XIX.—

Fig. 1.— Conidia of *Botrytis allii* n. sp.
Fig. 2.— Conidia of *Botrytis cinerea* Pers.
Fig. 3.— Germinating conidia of *Botrytis allii* n. sp.
Fig. 4.— Germinating conidium after 48 hours; showing cross connection and method of branching.
Fig. 5.— Cross connections in mycelium.

PLATE XX.—

Fig. 6.— Botrytis on onion bulb scale.
Fig. 7.— Portion of conidiophore. (Much enlarged; camera lucida drawing.)
Fig. 8.— Portion of tip of conidiophore from pure culture. Shows tendency toward more profuse branching in cultures.
Fig. 9.— Typical conidiophore from onion bulb. (Outlined with camera lucida.)
Fig. 10.— Conidiophores of onion Botrytis.
Fig. 11.— Botrytis mycelium in onion bulb scale.
Fig. 12.— Cross connections of mycelium in host tissue.
Fig. 13.— Cross section of germinating sclerotium.

PLATE XXI.—

Fig. 14.— Type of mycelium common in pure cultures.
Figs. 15–22.— Types of structures on mycelium in leaf tissue. (Camera lucida drawing.)
Figs. 23 and 24.— Developmental stages of the sclerotium.
Fig. 25.— Side view of holdfasts.
Fig. 26.— Type of heavy mycelium in pure cultures.
Fig. 27.— Incipient stages of sclerotium formation from organs of attachment.
Fig. 28.— Organs of attachment; view from beneath plate.
Fig. 29.— Organs of attachment, more enlarged; from beneath plate.
Fig. 30.— Appearance when tip of hypha touches glass.
Fig. 31.— Bottom view of holdfasts on glass plate.
Fig. 32.— Flattening of mycelium as it comes in contact with glass culture dish.

PLATE XXII.—

Figs. 33 and 34.— Typical budding, or sporulating filaments of onion Botrytis in media. (Camera lucida drawing.)

PLATE XXIII.—

Fig. 1.— Appearance of disease on white onions.
Figs. 2 and 3.— Appearance of the disease on necks of plants.

PLATE XXIV.—

Fig. 1.— Longisection thru diseased bulb.
Fig. 2.— Infection thru bottom of bulbs.
Fig. 3.— Botrytis sclerotia at necks of bulbs.
Fig. 4.— Sclerotial crusts on onion bulb.

PLATE XXV.— SCLEROTIA OF *Botrytis allii* n. sp.

PLATE XXVI.—

Fig. 1.— Two diseased and one healthy seed head.
Fig. 2.— Seed head (blasted) attacked by the fungus.
Fig. 3.— Effect of fumigation with formaldehyde gas on onions.

PLATE XXVII.— ONION BOTRYTIS CULTURES COMPARED WITH OTHER BOTRYTIS CULTURES.

PLATE XXVIII.—

Fig. 1.— Germination of sclerotium by forming stalk-like bodies.
Fig. 2.— Effect of fungal extract upon onion bulb scale.
Fig. 3.— Onion bulb injected with spore suspension.

PLATE XXIX.—

Fig. 1.— Sclerotial ring formation of Botrytis in petri dishes.
Fig. 2.— A very desirable type of onion storage house.

LIFE HISTORY STUDIES.

Primary infection of bulbs in the field.— From the early studies of the disease the writer was convinced that the mycelium was present in the bulbs when they were placed in storage where the disease developed with more or less rapidity depending upon the storage conditions. It has been found that approximately 80 per ct. of the diseased bulbs in certain instances showed the presence of the fungus at the neck, the remaining 20 per ct. showing the fungus upon the sides and bottoms of the bulbs. Examinations have shown that the fungus attacks the plants thru the necks and frequently thru the yellowing leaves where it works its way down into the neck of the bulb, remaining as a few mycelial threads or even as a mycelial mat between the outer three or four scales. Moreover, infection experiments, previously discussed, were made at the outset of the work, which yielded facts in accord with the above idea that infection takes place on the neck and passes to the bulb. In one plat of white onions under observation near East Lansing, Michigan, the neck-rot fungus was found fruiting abundantly on the necks and bulbs of the dying onions in October. A few weeks later when the bulbs were examined in storage they were found to be fairly well covered with the black sclerotia of the fungus.

That infection may also take place thru the bottom and roots of the bulbs is clearly evident. Here, the bulbs show abundant mycelium and masses of sclerotia at the bottom (Plate XXIV, fig. 2). As a rule the bulbs which show bottom infection are considerably under normal size and do not show the normal amount of roots attached when examined in storage. These bulbs invariably do not show the fungus mycelium in the upper parts of the bulbs. Experiments gave conclusive evidence that the fungus may pass from the soil to the bulbs and in such a manner as to escape the notice of the casual observer. It is not an uncommon occurrence to find onions which show the fungus at both the neck and bottom of the bulb. Infection may also take place thru wounds in the bulbs, and also thru onion-maggot injuries.

Infection of bulbs at harvest time.— When wet weather occurs during the harvesting and curing period, while the bulbs are drying in the field, a considerable amount of infection may take place thru the dying and drying leaves, especially when the bulbs are curing in the windrows. After being stored the neck rotting begins.

The writer visited a large storage house in central New York where 12,000 crates of onions were stored. According to an estimate made by the owner about one bushel out of every seven from certain lots was diseased. An examination of these diseased bulbs revealed a very striking condition in that nearly every bulb showed side infections, i. e., the disease showed at the sides of the bulbs and not at the necks or bottoms. This at once suggested a bruise or injury of some kind. Upon inquiry it was learned that an onion-topping machine had been used upon the greater part of this lot of onions. The bruising of the sides of the onions by the topping machine was the only explanation that could be offered for this condition of infection. The owner of the storage house had come to look with disfavor upon the topper because he had learned that machine-topped onions did not keep quite as well in certain seasons as hand-topped stock. He claimed that the topping machine bruised the sides of the large, sound bulbs, or as he expressed it: " it gives them quite a rap." A similar condition as regards side infections following the use of the onion-topping machine was encountered in one of the Allegan County, Michigan, onion regions. Here, the storage house operators claimed " that the bulbs were bruised by the machine and consequently rotted." On this account some growers object to the use of certain types of onion-topping machines because of the serious bruising of the bulbs. The writer believes that it will be found, thru a series of experiments which are now under way, that machine topping does not seriously injure the keeping quality of the onion if the stock is well cured. Very seldom does one find a bulb which has been injured during the process of topping when the work has been done by hand with shears. A certain amount of infection will take place thru wounds made by forks or shovels used in placing the onions in crates either before or after topping.

Secondary infection of bulbs in storage.— But very little secondary infection is believed to take place in storage, since the " contacts " (those healthy bulbs lying against diseased bulbs) very seldom become diseased unless the storage house is very damp and the dry outer skins become soft and full of water. The dry outer scales are usually the last to rot and the disease does not seem to spread from onion to onion thru the dry skins. Infection experiments which have been previously discussed show that the spores of the fungus when placed upon the dry onion scales, even under a bell jar, are unable to bring about infection.

Overwintering and dissemination of the fungus in the field.— From the outset of the investigation it was believed that the fungus causing the neck rot of onions was present in the soil and overwintered in the field. This idea was confirmed early in April, 1915, when the fungus was found fruiting upon some pieces of onion leaves in an onion field in central New York. Less than one-half mile from this spot there were several hundred crates of neck-rotted onions scattered in an old orchard less than forty rods from an onion seed field in which the fungus was found to be destructive to the seed crop later in the season. Altho the refuse onions had been dumped in the orchard early in December it was not difficult to find hundreds of bulbs which were covered with a profuse crop of conidia on this date. Again, on April 28 and on May 3, 1916, while going over an onion field in Michigan the writer found abundant spores being produced on refuse onion tops which had remained in the field all winter. Sclerotia were also found on some "scallions" or "thicknecks" (onions which develop no bulb, but have a long thick neck) which were discarded in the field at the time of harvesting. In both of these instances the disease was known to have been present and destructive in the fields the previous season.

As early as the middle of June the writer has observed the fungus attacking the stalks of the seed crop onions. Later in the season and even until late fall it was found upon onion bulbs and onion refuse left in the field, and again in the spring in the same field the fungus was found in its *Botrytis* form upon the old onion tops and necks left after the crop onions were topped the previous season.

It was found to be comparatively easy to isolate the onion Botrytis from the soil by using the method described by Jensen (1912). This author also gives a list (pp. 489–491) of seven species of Botrytis isolated from the soil.

Cultures made from the above-mentioned lots of material, supplemented by field observations and some experiments reported in this paper prove beyond doubt that the fungus may pass the winter on the diseased tops and necks of refuse onions as well as in the soil. The growing crop and also the seed crop may become infected from either of these sources. However, it is believed that the dumping of diseased refuse onions near seed fields becomes the most virulent source of infection for the onion flowers.

Dissemination in seed fields thru transplanting diseased bulbs.— Attention has already been called to the fact that the bulbs ("mother bulbs") used for the production of onion seed, when diseased, have a marked effect upon the amount of seed produced. There is great danger of transferring the disease to the seed fields by means of the infected bulbs. From the seed fields it readily spreads to the growing crop onions. Diseased bulbs usually produce a few small, erect, dark green leaves which die down very early.

Occurrence of the fungus spores on onion seed.— Since the fungus is seriously destructive on the flowers in certain seasons, a study was made of the possibility of the fungus being disseminated by means of spores adhering to the seeds. A quantity of seed was secured from a seed field in which the neck-rot fungus was destructively prevalent during the season of 1915. Quantities of this seed were then washed in distilled water and the washings poured into a centrifuge tube. After centrifuging at 2000 revolutions per minute for five minutes the clear liquid was decanted off and the sediment examined under the microscope for the fungus spores. In addition to a large number of spores of the Botrytis neck-rot fungus, there were found spores of the smut fungus (*Urocystis cepulae*), also spores of species of Cladosporium, Macrosporium, Fusarium and Rhizopus. In one instance the writer was fortunate in securing a small quantity of seed from the 1914 crop which was produced on the same field as the 1915 seed crop. The washings from this seed also showed the spores of the neck-rot fungus to be present and in addition spores of a species of Rhizopus.

RELATION OF PARASITE TO HOST[1]

At the beginning of the investigation it was noted that a peculiar relation existed between the host and parasite. A macroscopical examination of the leaves, necks, and bulb scales of the host, when attacked by the fungus, reveals a discoloration of the tissues, and very often a boiled or water-soaked appearance somewhat in advance of a point where the fungus may be seen fruiting.

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[1] An abstract of this section was read before the Botanical Section of the Michigan Academy of Science in its Twenty-first Annual Meeting at Ann Arbor, Michigan, March, 1916.

HISTORICAL REVIEW.

Kühn (1858) in speaking of the penetration of the host tissue by fungous hyphae mentions it as a process of boring thru ("Durchbohrung").

De Bary (1863, 1866, and 1884) mentions the process of the penetration of the host by fungi, but states that he had no knowledge of the forces causing this boring into the tissue. This author (1884) working with *Sclerotinia libertiana,* appears to be the first to report that a substance was produced which discolored the host tissue, plasmolyzed the cells, and later killed them. De Bary was able to isolate this thermolabile substance and ascribed its cell-wall-dissolving action to an enzym, altho in one place he suggested the possibility of the toxic action being due to oxalates which he found encrusting the mycelium. It appears that he later changed his ideas in regard to the action of the oxalates since solutions of oxalic acid did not give such markedly destructive effects.

Ward (1888) in a searching investigation of a lily disease caused by a Botrytis found that the tips of the fungus hyphae secreted a substance which "has the property of swelling and dissolving the cellulose cell walls." This author ascribed this action to a soluble ferment.

Kissling (1889), using methods similar to those employed by Ward, found that *Botrytis cinerea* formed an enzymic poison which kills the invaded tissue of the host. Apparently, his work confirms that of De Bary.

Kean (1890) also noted the "glairy fluid" exuding from the tips of hyphae of the same Botrytis growing upon *Lilium* sp. as was noted by Ward. This author was also able to demonstrate the softening and decomposition of pieces of healthy sweet potato tissue by the expressed and filtered juice from a rotting sweet potato which had been attacked by *Rhizopus nigricans.* He then obtained a precipitate from this filtered juice by the usual alcoholic precipitation, which carried with it the active principle of the extract. Kean was also able to get similar results and a similar substance from Ward's lily Botrytis and from a Botrytis growing upon *Sedum telephium.*

Nordhausen (1899) working with *Botrytis cinerea* found an enzym present which behaved similarly to that of De Bary's Sclerotinia.

Behrens (1898) working with *Botrytis cinerea* among other fungi, found in addition to a pectin-dissolving substance, a thermostable toxic body which had a marked action upon the host tissue. This author concludes that Botrytis is capable of dissolving cellulose.

Smith (1902) from his work with *Botrytis cinerea* reports dissolving action by this fungus upon the middle lamella. This action he found to occur even after the fungal extract had been boiled. This author believed the dissolving of the cell wall to be due to oxalic acid, but does not give his methods for determining the presence of the acid.

Schellenberg (1908) studied the action of various fungi upon hemicelluloses and concluded that the penetrating and dissolving power of *Botrytis cinerea* is due to its ability to dissolve hemicelluloses. He believed the middle lamella to be composed of hemicelluloses.

Jones (1909) in his painstaking work with the cytolytic enzyms produced by *Bacillus carotovorus* and other soft-rot organisms (forty-five from different sources) shows conclusively that a middle-lamella-dissolving enzym is produced. This author gives a complete resumé of the literature dealing with phyto-pathogenic bacteria and their relation to the host tissue.

Bruschi (1912) grew *Monilia cinerea* in a medium containing the flesh of the plum, and after forty-eight hours noted that all of the cells were separated one from another, therefore he concludes that the enzym pectinase was produced by the fungus. He was unable, however, to isolate a cellulose-dissolving enzym.

Peltier strongly emphasizes the two-fold aspect of the disintegration of the host tissue by a Botrytis appearing as a parasite on peppers. This author believes that some substance is secreted by the fungus which kills the tissue in advance of the mycelium.

Brown (1915, 1916), whose work will be reviewed below, appears to be the first to attempt to obtain the active principle from the young fungal elements of a Botrytis. This investigator made a powerful extract from the germ tubes and showed that the only active substance present in the fungal extract is an enzym which appears to be responsible for the two-fold action of the plant tissue.

Valleau (1915), working with *Penicillium expansum*, performed some experiments which strongly indicate that that fungus secretes a very active middle-lamella-dissolving enzym, pectinase. This author was unable to extract the enzym pectinase from an extract of the fungus *Sclerotinia cinerea*, or to demonstrate its presence in rotting fruits.

Because of the peculiar mode of action and method of infection of the onion fungus a study was made of some of the striking features of its method of parasitic attack upon the host.

PATHOLOGICAL HISTOLOGY.

A microscopic examination of sections of a large number of the diseased bulbs from storage houses showed that invariably the host tissue was killed somewhat in advance of the tips of the hyphae of the fungus. This was very easily shown by cutting small blocks containing both healthy and diseased tissue from the affected bulbs. These blocks were plunged into 95 per ct. alcohol for slightly less than an hour for most of the blocks. Free-hand sections containing both diseased and healthy tissue were stained in eosin for a few minutes, then decolorized in acid alcohol for an instant. These sections show the injured cells to be considerably darker than the healthy cells. The fungus is differentiated from the host cells which remain unstained. The diseased areas show plainly that there is a toxic or lethal action, or both, in advance of the hyphae. The cells are discolored, the degree of the discoloration being a possible index of the severity of the toxic action. Following discoloration, the cells are plasmolyzed, the contents become granular and later the cell walls collapse, this being regarded as a lethal action. It seems that the middle lamella is readily dissolved by the action of the fungus which later sends its hyphae between the cells, directly thru the disorganized cell walls, or across the intercellular spaces and very often directly from one cell into the next (Plate XX, fig. 11). The host tissue may be found as a disorganized mush of a firm consistency filled with the fungus mycelium; or the cells, in some of which the distorted protoplast may still be seen, may appear to be held in place by the mycelium. From this effect on the host it would appear that a middle-lamella-dissolving enzym is produced. A consideration of a few typical cases will serve to make these points clear. Figure 3, Plate XXVIII, shows one of a number of sound bulbs which were injected with a spore suspension of the onion Botrytis spores in distilled water, using a hypodermic syringe. The point of inoculation is at A. From this point the heavy Botrytis mycelium was found extending out in all directions and gradually decreasing to the point B where none was to be found. From B to the margin or border of the diseased area the tissue was discolored, and the cells were plasmolyzed and loosened.

A longisection thru a diseased bulb is shown in Figure 1, Plate VI, between the scales near the neck and extending down nearly to the center of the bulb one can see the mycelium woven together in the form of a dense weft or crust. All of the scale tissue below, altho no mycelium was found to be present, was discolored and the cells were broken down. The sprout in the center has been attacked at the upper end where it is in contact with the diseased scales and shows some discolored and softened tissue.

Stevens (1916) in a recent paper describes an almost identical condition as found from a study of the pathological histology of strawberries affected by *Botrytis* sp This author concludes that the fungus is evidently capable of not only dissolving the middle lamella but of penetrating the cells themselves.

CELL WALLS OF THE HOST.

Since the fungus showed a very marked action upon the host-plant cell walls a brief study was made of the tissue of the necks and bulbs.

Tests for cellulose were positive in all cases, the best reactions being secured with cuprammonia or Schweizer's reagent in which the cellulose is soluble. A concentrated chloriodid of zinc solution, acting directly upon the sections and giving them a violet color, was also successfully used.

The middle lamellae of the host cell membranes were shown to consist of pectic acid, or its insoluble salts, by treating thin sections for twenty-four hours with a mixture composed of one part hydrochloric acid and four parts 95 per ct. alcohol. At the end of this time they were washed with water, then treated with a 10 per ct. solution of ammonia for a short time when the sections could be easily separated into their constituent cells by gentle pressure. When these sections were stained with methylene blue for a few minutes after treatment with the acid alcohol the middle lamellae stained very deeply. The results of these and other tests applied agree with the statements of Mangin (1892, 1893) that pectose is very pronounced in the cell walls of young tissue, but in older tissue calcium pectate comprises the greater part of the middle lamella.

PRODUCTION OF OXALIC ACID BY THE FUNGUS.

Preliminary to a study of the enzyms produced by the fungus it seemed necessary to make a study of the possibility of oxalic acid being the toxic substance produced.

De Bary (1886), in his study of the fungus *Sclerotinia libertiana*, reported that the hyphae were often coated with crystals of oxalates. He found as high as 0.39 per ct. oxalic acid in one sample of his fungus. It appears that this author discounted the possibility of oxalic acid being the toxic substance since he was unable to secure the same effect upon plant tissue with solutions of oxalic acid as was obtained with the fungus or an extract of the fungus cultures. De Bary was unable to demonstrate any free oxalic acid.

Ward (1888, p. 336) reports the presence of minute crystals upon some of the conidiophores in his Botrytis cultures upon raisin extract.

Wehmer (1891), in a valuable series of articles reporting his work upon the production of oxalic acid by a number of fungi, points out that the fungi studied vary as to their ability to produce this acid, depending upon their metabolism as influenced by different factors which he gives. He holds that oxalic acid is a type of excretion.

Nordhausen (1899), working with a Botrytis, permitted the hyphae of this fungus to grow thru pieces of the epidermis of Allium which had been previously stained with congo red. The absence of a blue acid dye around the penetrating hyphae was taken as evidence of the failure of the fungus to excrete an acid, at least one as strong as oxalic acid.

Smith (1902), working with *Botrytis cinerea*, stated that he found over 20 per ct. oxalic acid in the extracts of mycelium from old cultures. This author was inclined to believe that oxalic acid is responsible for part of the action upon plant tissues. He was unable to detect any difference between the action of boiled and unboiled mycelial extracts upon petioles of lettuce leaves. Working with solutions of oxalic acid he reported a bleaching effect from the acid, which was noticeable to the naked eye.

Peltier (1912), in his work with a Botrytis, was unable to detect any difference between the boiled and unboiled extracts. Applying several delicate tests which, however, he does not outline, he was unable to detect the presence of oxalic acid. This worker also tested the action of different concentrations of oxalic acid upon petioles of lettuce and found it to be somewhat like that of the mycelial extract, except that a bleaching of the tissues took place. He found this to be true of the organic acids and therefore concludes from his experiments that it is not oxalic acid, but may be some undetermined organic acid.

Cooley (1914), who gives his method of analysis, found oxalic acid to be produced in appreciable amounts by *Sclerotinia libertiana* when cultured on certain fruits.

Valleau (1915) studied the middle-lamella solvents concerned with the resistance of plums to brown-rot and reports some very interesting results with oxalic acid. He immersed blocks of tissue from various species of plants, among them onion and potato, in 0.015, 0.062, and 0.125 per ct. solutions of oxalic acid for stated periods of time. The various tissues showed varying degrees of resistance to the action of the acid, but all those affected showed that the softening was due to the solution of the middle lamella. The onion tissue softened in the 0.125 per ct. solution, while the potato did not soften even in a 0.25 per ct. solution. Valleau noted a marked bleaching effect produced by the acid and concludes from his experiments that oxalic acid " is not the sole toxic substance produced."

Brown (1916) found no evidence, at the end of twelve hours, of the action of a N/40 solution of oxalic acid or of N/20 potassium oxalate when placed on leaves of bean, or the petals of rose. He found, however, that the fungus (*Botrytis cinerea*) excreted an oxalate into the " infection drop," but that M/800 represented the upper limit of concentration. This author states that infection by the fungus could take place even tho the presence of a soluble oxalate was excluded.

Oxalate crystals in media.— While examining the medium directly beneath some cultures of the onion Botrytis growing on lettuce-leaf agar plus peptone (1 per ct.), the writer found a large number of crystals distributed thruout the substratum. These tetragonal pyramids had every appearance of calcium oxalate crystals. When treated with concentrated hydrochloric acid they dissolved slowly. At first it was believed that these were present only in the agar cultures containing peptone since Emmerling (1903), from his study of oxalic acid formation by certain fungi, stated that abundant production of oxalic acid takes place on substances containing peptone. A further examination of the substratum from cultures of the onion Botrytis and ten other strains or species of Botrytis from as many hosts, and growing upon onion agar, showed the presence of these crystals. Examinations of lettuce-leaf agar cultures to which no peptone was added showed these crystals to be present. The cultures examined varied in age from 30 to 60 days. When carefully examined microscopically and tested microchemically these crystals appeared to possess all the properties and characteristics ascribed to crystals of calcium oxalate by Zimmermann (1893), Tunmann (1913) and Molisch (1913). Examinations made by Mr. O. B. Winter, Assistant Chemist of the Michigan Experiment Station, using the polarizing microscope, showed them to be of the monoclinic or tetragonal system. Hence, his observations confirmed those of the writer, since calcium oxalate possesses just such properties.

Oxalic acid in the diseased tissues.— In order to determine whether any free oxalic acid could be detected in the diseased, decaying onion bulbs, microchemical tests were made using calcium nitrate which showed no crystals of calcium oxalate to be formed. Other microchemical tests for oxalic acid as given by Zimmermann, Tunmann, and Molisch gave negative results.

Oxalates in the diseased tissues.— To ascertain whether any oxalates were produced by the fungus upon its host, chemical analyses were made of diseased and healthy onion bulbs. The analytical method used by Wehmer (1891) was employed. The soft, decayed tissue of onion bulbs overrun by the onion Botrytis was carefully cut away from healthy tissue. This material was then digested in cold hydrochloric acid, using in each lot 100 grams of the diseased onion tissue to 150 cc. of the acid. Controls were prepared by using 100 grams of healthy onion bulb scales sliced into 150 cc. of the acid. These lots were allowed to digest for 24 hours when the analyses were made.[1] The oxalic acid or its soluble oxalate was precipitated out as a calcium salt, which was filtered off, dried to constant weight and weighed. Results as follows:

Healthy onion tissue showed............	0.0050 per ct. oxalic acid
Diseased onion tissue showed............	0.0098 per ct. " "

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[1] The determinations for oxalic acid were made by Mr. O. B. Winter, Assistant Chemist of the Michigan Experiment Station, to whom acknowledgments are due.

The analyses of liquid culture media yielded some very interesting results. Two hundred grams of clean onion bulbs were added to one liter of distilled water. This was then autoclaved for twenty minutes at fifteen pounds pressure when the juice was pressed out and 500 cc. placed in each liter Erlenmeyer flask and again autoclaved for 15 minutes at 15 pounds pressure. Some of the flasks prepared in this manner were inoculated with the onion Botrytis spores, the remainder was retained as checks. The cultures were 56 days old when the analyses were made. The liquid from beneath the fungous crusts was filtered off and the determinations made exactly as indicated above. The results are as follows:

Autoclaved onion juice, uninoculated......0.0040 per ct. oxalic acid
Autoclaved onion juice, from beneath
growth..........................0.0115 per ct. " "

The results of the determinations show that the onion Botrytis, when grown upon liquid media or upon onion bulbs, produces appreciable amounts of oxalic acid.

Titration of the liquid media.— At the time the determinations for oxalic acid were made, 5 cc. portions of the liquid from beneath the cultures were removed and added to 45 cc. of distilled water. This was then titrated with N/20 NaOH using phenolpthalein as an indicator. It required less than 0.1 cc. of the N/20 NaOH to neutralize the free acid in the juice; in fact, one drop of the N/20 alkali brought a distinct pink color. The juice was recorded as being +1° acid on Fuller's Scale. This would seem to indicate that there was but little free acid present.

Acidity of sound and diseased onions.— In the hope that the determination of the acidity of the diseased and sound onion bulbs would prove of value the following tests were made: Thirty-gram samples of the sound and diseased onion tissue were titrated with N/1 NaOH. The sound tissue was taken from several large healthy onions. The diseased tissue was taken from a number of onion bulbs attacked by the onion Botrytis. These lots of tissue were ground to a pulp in a mortar and then washed out with a large volume of distilled water into a large evaporating dish. The pulp and the water were thoroly stirred after which the titrations were made with N/1 NaOH using phenolpthalein as an indicator. The results of the titrations given in terms of cubic centimeters of normal alkali required to neutralize the acid in 30 grams of pulp are as follows:

30 grams of sound onion tissue required.......	1.2 cc. N/1 NaOH.
30 " " diseased " " " "	1.6 " "

In another series of tests at an earlier date the 30 grams of sound tissue required 1.3 cc. of normal alkali to neutralize the acid present.

From the results of these titrations it seems safe to conclude that the acidity of the tissue slightly increases following the attacks of the fungus. It is to be noted, however, that the bulk of sound tissue taken for titration is larger than the bulk of diseased tissue, and that the sound tissue seems to contain more juice than the diseased tissue.

Action of oxalic acid solutions upon plant tissue.— In view of the somewhat conflicting results concerning the action of oxalic acid upon various plant tissues and to study the possibility of this acid being a factor in the toxic action of the fungus studied, the following experiments were carried out: Various concentrations of oxalic acid were prepared using Kahlbaum's pure chemical. The concentrations in four cases were the same as those found in the samples analyzed and reported on pages 419 and 420. The action of the following concentrations was tested: 0.2000, 0.1260, 0.0115, 0.0098, 0.0050, and 0.0040 per ct. Pieces of onion-bulb scale, and potato-tuber tissue of the same thickness, namely 0.5 mm. were placed in these solutions and in beakers of distilled water for controls. No appreciable changes in any of the tissues could be detected until about six hours had passed when it was apparent to the naked eye that the potato-tissue discs in the four higher concentrations were bleached out whiter. No changes could be detected in the 0.0050 and 0.0040 per ct. solutions, except that the tissue discs were not as turgid as those in the water controls. The onion tissue did not show any noticeable changes. At this time the potato discs from the 0.2000 per ct. solution only, when compared under the microscope with the discs from the water, showed what appeared to be a reduction in size of the starch grains. No change in the cell walls could be detected.

In twenty-four hours the potato discs in the 0.2000 and the 0.126 per ct. solutions showed almost complete loss of coherence, that is to say, all of the cells were easily pulled or pressed apart. The cells floated free and rounded off, but the walls were apparently unaltered. The potato discs from the water controls were turgid, brittle and pulled apart with a cracking, splitting sound. All of the potato discs in all of the acid solutions showed a change in the starch grains,

i. e., these appeared to be smaller and present in larger number. The discs were denser when viewed under the microscope and compared with those from the water controls, apparently due to the change in the starch grains. At the end of the twenty-four hours the onion discs in only the 0.2000 and the 0.126 per ct. solutions showed a slight loss of coherence. None of the cells floated free as was true of the potato discs. No change could be detected in any of the other solutions except that all showed a bleaching or whitening effect upon the tissue.

At the end of forty-eight hours the potato discs in the 0.2000 and 0.126 per ct. solutions fell apart very easily, the cells appearing free with the walls intact. The discs in the 0.0115 per ct. solution showed a very slight loss of coherence, i. e., the tissue pulled apart, or was very slightly more easily torn than the tissue discs in the water controls. The potato discs in the 0.0098 per ct. or lower concentrations, aside from the whitened appearance, were in no way different from the discs in the water controls which retained their clear color and their turgidity. The onion discs from the 0.2000 and 0.126 per ct. solutions at the end of the forty-eight hour period were quite easily pulled apart, but they did not seem to have lost coherence of tissue to as great an extent as the potato discs. The onion tissue in the 0.0115 per ct. solution had unmistakably lost a very slight amount of tissue coherence, but the discs in none of the concentrations below 0.0115 per ct. showed any change except the bleaching effect which was evident in all of the concentrations. The discs in all of the solutions were flaccid, i. e., they were not as turgid as those in the water controls.

Plainly, at the end of forty-eight hours certain concentrations of the oxalic acid solution caused a solution or softening of the middle lamellae of the cell walls, the remainder of the cell walls remaining intact. All of the concentrations used brought about a bleaching or whitening of the tissues exposed.

The results from these and similar tests agree in many particulars with those secured by the investigators whose work has been reviewed above. All are agreed that oxalic acid solutions caused a bleaching of the tissues exposed. This was also true of other acids used. Valleau (1915) failed to get softening of potato tissue in a 0.25 per ct. solution. He stated, however that he used small blocks of the tissue. This author reported a slight softening of the onion tissue in a 0.125 per ct. solution of oxalic acid. This compares closely with the writer's observations.

In the case of all of the tissue discs exposed to the action of oxalic and other acids, and examined microscopically by the writer, the softening of the tissue appeared to be due to a solution of the middle lamella. At all events, with the possible exception of such solution of the middle lamella, the writer is unable to find, in the action of the oxalic acid, any of the pathological changes taking place when the tissues are acted upon by the fungus itself. Even tho appreciable amounts of oxalic acid are formed by the fungus it cannot be regarded as the toxic substance secreted by the fungus. Furthermore, it is believed by the writer that it plays no appreciable part in the disintegration of the host tissue by the actively advancing mycelium.

PRODUCTION OF AN ENZYM BY THE FUNGUS.

Action of extracts from old mycelium.— Ward, Kean, Kissling, Smith, Nordhausen and Peltier made extracts from old Botrytis mycelium and noted its effect upon various plant tissues. Ward (1888), in studying the action of the lily Botrytis, made flask cultures of the fungus on prune juice. The mycelial crusts were removed at the end of three weeks and crushed in a mortar with a little distilled water. The liquor was then divided into two lots, one of which was boiled. Slices of lily tissue added to the respective solutions showed a marked swelling of the cell walls in the case of the unboiled extract, while in the boiled extract no change was observed.

Working along the line suggested by Ward (1888, p. 351) the writer made solutions of onion and prune juice. Five hundred cubic centimeters of the juice were placed in liter flasks which were then plugged, sterilized, and later inoculated with spores of the onion Botrytis. At intervals, ranging from four to twelve weeks, the mycelial crusts were removed with a sterile glass rod and thoroly ground in a sterile mortar with a very small quantity of washed, sterilized quartz sand. After grinding, enough sterile distilled water was added to give the mass fluidity when it was allowed to stand for an hour. The thick liquid, which was of a straw color and which had a sweet, sickening odor, was then filtered thru sterile filter paper into sterile glass beakers. Some of the beakers of liquid were placed over a gas flame and brought to a boil for one minute.

When the solutions were cool, discs of potato tuber tissue and thin slices of onion scale obtained aseptically were added. Checks were run by adding similar pieces of tissue to beakers containing sterile distilled water. At the end of three hours no change in the appearance of the pieces of tissue could be detected, but at the end of ten hours a very striking contrast between the tissue discs in the various solutions was plainly evident. The cell walls of the discs from the unboiled extract were greatly swollen in some places and laminated. They were not at all refractive. It was very difficult to detect the adjoining cell cross walls in some cases. The onion tissue cells did not show this change to as great an extent as did the potato tissue cells. No change could be detected in the tissue discs from the boiled extract, or from the distilled-water checks. These results agree with those obtained by Ward and De Bary. The experiments were discarded at the end of twelve hours because of possible bacterial action altho the presence of bacteria could not be detected microscopically at this time.

It was noted that when the fungal crusts were removed from flasks of liquid nutrient solutions an abundant growth did not reappear on the medium, altho numerous spores were present. Experiments with spores in hanging drops of the solution from the cultures showed that germination was retarded. This appears to be in accord with the observations of Lutz (1909) who found that *Botrytis cinerea* produced certain substances in nutritive solutions which retarded the germination and growth of that species of fungus. The products formed had many of the characteristics of enzyms.

Ward (1888, p. 348) tested the action of the liquid filtered from beneath the dense mycelial crusts of Botrytis growing on three different solutions and found that in hanging drops the unboiled filtrate had caused a distinct swelling of the cell walls of sections of lily bulbs. The writer made similar tests with the liquid secured from beneath dense mycelial crusts of the onion Botrytis growing upon a weak solution of onion and prune juices. Using a sterile pipette, quantities of the clear liquid were removed from the cultures at various ages and passed thru sterile filter paper into sterile beakers. Potato- and onion-tissue discs were then added to the boiled and unboiled filtrate and also to checks of sterile distilled water. The discs were removed and examined at the end of 6, 12, 24, 36, and 48 hours, but at the end of this time the writer was satisfied that very little, if any, change could be detected in any of the solutions with the exception of the unboiled filtrate in which the tissue discs showed a certain amount of cell plasmolysis and possibly some change in the cell walls.

In this connection the results of the work of Reed (1912), Dox (1910) and Franceschelli (1915) are very valuable and interesting, since they also show that the mycelia of certain fungi possess active principles.

Action of droplets exuded from upper surface of cultures.— De Bary (1886) studied the pathogenic action of *Sclerotinia libertiana* and found that the active principle of the droplets of liquid exuded from the young sclerotia were thermolabile and therefore concluded that it was of the nature of a ferment.

Jones and others (1912) report the appearance of drops of a clear, brownish liquid exudate upon the surface of vigorous-growing cultures of *Phytophthora infestans*. These authors suggest that this is a normal bleeding process comparable to that observed by Pfeffer (1900). A quantity of the exudate was titrated to phenolpthalein and found to be slightly acid in reaction,— less so than the normal potato juice.

Ward (1888, p. 339) reports the extrusion of drops of liquid from the tips of hyphae at the margin of large mats of Botrytis mycelium grown on Pasteur's solution. He was able to obtain some definite reactions from these drops and concluded that they are made up quite largely of a soluble ferment which has the property of swelling and dissolving cellulose cell walls. The writer has observed drops of liquid, which may be very similar to those noted by Ward, extruded upon the upper surface of cultures of the onion Botrytis growing upon bread soaked with prune and onion juices. Similar drops were also observed upon onion-agar slants covered with a dense growth of the fungus. These drops varied in size from 1 to 3 mm. in diameter. They were of a clear, amber color, and rather thick and viscid. When tested with Millon's reagent negative results were secured. When placed upon blue litmus paper a strong acid reaction was indicated. In order to test the action of these drops upon the onion tissue some of them were taken up with a sterile 1 cc. pipette and placed upon the inner surface of onion bulb scales obtained aseptically and placed in sterile preservation jars, kept moist by the usual devices. At the end of three days the tissue of the onion bulb scales upon which the drops were placed showed that dissolution of the middle lamella of the cells had taken place directly beneath the liquid. The cells were loosened and the wall wrinkled and folded. The contents of the cells were plasmolyzed and the tissue was discolored over an area of about 5 mm. around the edge of the drops. Sections thru these softened areas showed no fungus mycelium to be present. Checks were run by placing sterile distilled water on onion bulb scales similarly treated. These remained unchanged.

Action of extract from diseased bulbs.— De Bary (1886), Kean (1890), and more particularly Behrens (1898) studied the extract from diseased plant parts. All of these investigators demonstrated the activity of the extracts secured.

Voglino (1903) filtered thru a Pasteur-Chamberland filter the slimy mass obtained by squeezing garlic bulbs attacked by *Sclerotium cepivorum*. The filtrate when placed on healthy bulbs caused destruction of the external tissues. This author finds this to be due to a "special substance" which infiltrates into the healthy tissue, thus separating the cells one from another.

To determine whether an extract from diseased onion bulbs had any effect upon healthy host tissue the following experiment was performed: A large number of onion bulbs attacked by the Botrytis were secured and the soft, diseased tissue cut off and placed in a mortar where it was ground to a pulp. Some care was exercised in cutting the tissue to discard all of that badly overrun by the fungus. The pulp was then transferred with the contained liquid to a bulb pipette which was sealed down to a Chamberland filter of the pencil type fitted to a suction flask. The liquid was then drawn down into the flask where it was permitted to digest for thirty-six hours. At the end of this time it was found to be sterile by plating out in media, and small quantities of about 1 cc. were withdrawn with sterile pipettes and placed upon onion scales. The onions from which the scales were secured were first cleaned by removing the outer scales, then immersing for two minutes in mercuric chlorid followed by sterile distilled water. The outermost scales were then peeled off and the inner ones secured as aseptically as possible with flamed forceps and scalpel and placed, concave side up, on crystallization dishes in large, sterile preservation dishes. Checks were run by placing sterile distilled water on some of these onion bulb scales. At the end of forty-eight hours, when the greatest change was evident, the scales to which the extract had been added showed the tissue beneath the liquid to have a dark, water-soaked appearance thru the entire thickness of the scale. Fig. 2, Plate X, shows a photograph of one of these scales, the under or convex side of the scale being shown here. Sections thru the spots over which the liquid had remained showed the cells to be loosened and plasmolyzed, and, in some cases, the walls were much laminated and folded. When a piece of this tissue was placed on a slide and covered with a cover glass, gentle pressure separated the cells very readily. The checks remained unchanged. No evidences of bacterial action could be detected with the microscope. It was plainly evident that a substance obtained from the diseased onions passed thru the filter used, and had a marked influence upon the host tissue.

In another series of experiments about 5 cc. portions of the extract, which had passed thru the filter, were placed in sterile Erlenmeyer flasks. To these were added discs from onion scales obtained aseptically. The discs were 1 cm. in diameter and the thickness of the bulb scale. Checks were run by using sterile distilled water in place of the extract on another lot of discs. In twenty-four hours the discs in the extract showed marked loss of coherence, that is to say, when the discs were pulled or pinched between the fingers the tissue appeared to be softened. Microscopic examination confirmed the macroscopic observations in that it showed the cells to be loosened along the line of the middle lamella.

Using the filtered extract noted above, about 1 cc. was injected into each of three healthy onion bulbs. Five days later when the bulbs were cut open the results appeared to be negative with the exception of one bulb which showed a marked discoloration of the tissue along the track of the needle.

The results of these experiments appear to give strong evidence of the production of an enzym or enzyms by the fungus.

Action of the extract from young mycelium.— In spite of the fact that the foregoing results point to the constant production of an enzym the active principle displayed by the products prepared from the old mycelium were not as active as was believed to be the case with the young, advancing hyphae of the fungus. The logical procedure, then, appeared to be the preparation, if possible, of an extract of the young elements of the fungus, since Dox (1910) stated that the time to prepare the intracellular enzyms is before fructification.

Brown (1915), in his painstaking study of the physiology of parasitism of *Botrytis cinerea*, immersed thin discs of tissue from various plants in a strong extract made from the germ tubes of that fungus. In his work he was able to demonstrate that the extract, which he inactivated by heating, by mechanical shaking, and by neutralization with alkali, possessed a two-fold action on the plant tissue. It produced a macerating action by dissolving the middle lamella, and produced death of the cell by its action on the protoplast. These two actions are thought by this author to be possessed by the enzym found in the extract.

Stevens (1916) in making a careful study of the pathological histology of strawberries affected by a species of Botrytis accepts Brown's conclusions as to the conditions found in the cells attacked by the strawberry fungus.

The method as employed by Brown was found to be applicable to the problem at hand and was followed, with some modifications. In short, the method used by the writer was as follows: An abundant supply of spores was secured by growing the fungus upon onion agar[1] which was found to be the most satisfactory of the various kinds of media tried.

Botrytis spores for inoculation were secured from cultures made from isolations from diseased onions from storage houses in New York. Previously inoculated tubes of onion agar were poured into the ordinary petri dishes. A copious spore production took place upon such plates and in eight to ten days the spores could be readily removed by covering the plates with sterile distilled water, and then by the use of a sterile glass rod bent at a slight angle at one end, the spores together with a slight amount of the aerial portions of the cultures, were brushed off. This material was then filtered thru a piece of sterile linen having 19 threads per linear centimeter, into a sterile centrifuge tube. The spores readily pass thru with the water leaving the fungal debris upon the filter. After centrifuging for two minutes at a speed of 2000 revolutions per minute the spores were thrown to the bottom of the tube where the wet volume was noted. The water was carefully siphoned off and the centrifuged spores were suspended in a nutrient solution made by autoclaving cleaned onions for fifteen minutes and then using the sterile juice. The spores were suspended in this onion juice at the rate of 0.5 cc. wet volume to 50 cc. of the nutrient liquid. This spore suspension was then sown on circular glass plates eight inches in diameter, supported on three large corks in large, sterile, deep, culture dishes. A small amount of water was placed in the bottom of the culture dishes. After being carefully leveled and sterilized the glass plates were ready for seeding. This was done by distributing 5 cc. of the spore suspension very evenly over the plates by the use of a sterile, fine pointed, 5 cc. pipette and a sterile glass rod bent at a slight angle at one end. After thirty-six hours at a temperature of 22° C., a copious spore germination had taken place producing a continuous weft of germ tubes. This weft was readily peeled or scraped off with a sterile microscope slide and thrown upon a sterile filter made of a linen handkerchief placed over a ring stand. After being vigorously washed under the water tap for one-half hour to remove all of the culture medium, ungerminated spores, etc., it was treated to four washings with sterile distilled water, squeezed as dry as possible, then spread out upon sterile pieces of filter paper which were placed upon squares of the kind of netting known as hardware cloth and lowered into desiccators containing calcium chlorid. In from twenty-four to thirty hours this fungus material was dry. It was then scraped off, carefully weighed, and mixed with an equal weight of sterile, washed, clean, dry sand. This material was then ground to a fine, light gray powder. This powder had a distinct odor of fresh mushrooms. In order to obtain an active extract, this spore material or powder was extracted in the proportion of 0.2 grams of the powder to 3 cc. of sterile distilled water. After one hour's extraction the liquid was cleared by centrifuging for three minutes at 3000 revolutions per minute, decanted, and again centrifuged and decanted. This gave a liquid having a light straw color and an odor resembling mushrooms.

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[1] See footnote page 355.

Tests were then made with this crude fungal extract as to its ability to destroy the coherence of onion and other plant tissues placed in it for various lengths of time. Brown (1915), in his investigations, tested the degree of coherence by pulling the discs of tissue with the hands. In order to eliminate the personal equation which occurs when the coherence is tested in this manner and to obtain some definite numerical values for strength of tissue affected by the extract at stated intervals of time, a chemical balance such as is used for rough weighings was utilized. The pan was removed from one side of the balance and a contrivance consisting essentially of two clamp clothes pins, the jaws of which were covered with corrugated rubber, was substituted. After being counterpoised, the tensile strength of pieces of onion and potato tuber tissue of uniform thickness and width could be determined with a surprising degree of accuracy in this crude piece of apparatus by pouring sand into a receptacle on the opposite pan, the sand being weighed after each test. Pieces of tissue for the purpose of these tests were very easily prepared by cutting strips of onion leaves 7 mm. in width, also by cutting the same width strips of the thin membrane found between the scales of the onion. Strips of potato-tuber tissue of uniform thickness (0.5 mm.) and width (7 mm.) were also used. Two safety-razor blades securely clamped to a piece of hard wood 7 mm. in thickness served very nicely to cut the strips of uniform width. A sliding microtome was used to cut the desired thickness of tissue. The pieces of tissue were freed from contained air by injection, under the air pump, with water. They were then placed in the extract and removed at intervals and tested for coherence. Checks were provided in all cases by using sterile distilled water in place of the extract on part of the tissue discs.

Effect on tissue strips. — After a considerable number of preliminary experiments, all of which gave uniformly satisfactory results, the following experiment was performed. A detailed record of this experiment will suffice to make clear the methods used and aid in interpreting the results secured. Values of tests for coherence are recorded in round numbers in grams, i. e., in terms of grams of sand required to break a certain width and thickness of tissue after being acted upon by the fungal extract for a given length of time. Where time permitted of the securing of more than one value for a tissue the average of the value is given.

A series of tissue discs in quantities of the boiled extract was carried along with the above series. These discs showed approximately the same values at the end of the twelve hours as at the beginning. They were practically the same as the checks. All of the tests were discontinued at the end of the twelve hours since loss of

TABLE IV.— EFFECT OF THE FUNGAL EXTRACT UPON ONION-LEAF AND SCALE-MEMBRANE TISSUE, AND UPON POTATO-TUBER TISSUE.

TIME.	PLACED IN STERILE DISTILLED WATER.			PLACED IN THE FUNGAL EXTRACT.		
	Onion-scale membrane.	Onion-leaf tissue.	Potato-tuber discs.	Onion-scale membrane.	Onion-leaf tissue.	Potato discs.
	Gms.[1]	*Gms.*[1]	*Gms.*[1]	*Gms.*[1]	*Gms.*[1]	*Gms.*[1]
Initial reading, average....	78	365	122	77	363	127
30 minutes...............	79	370	115	79	360	113
45 "	71	......	......	83	......	....
1 hour..................	76	345	130	80	355	101
1 " 15 minutes.......	80	......	......	67	......	100
1 " 45 "	69	340	123	57	340	95
4 hours.................	76	350	125	34	301	33
6 "	76	355	134	30	210	27
12 "	70	338	120	[2] Soft	200	[2] Soft

[1] Weight required to destroy coherence of strips 7 mm. wide of onion leaves, of membrane separating onion bulb-scales and of potato-tuber tissue 0.5 mm. thick.

[2] By "soft" is meant that the tissues had so lost coherence that they could not be handled or placed on the balance to be tested as to their strength.

coherence had occurred in some of the tissues before the expiration of this time. The possibility of bacterial action appears to have been eliminated in this period of time, since no bacteria were revealed by the aid of the microscope.

The writer is satisfied that complete loss of coherence in many instances took place in less time than 12 hours; however, a weighing of approximately 20 grams at six hours was the least that could be satisfactorily made with these tissues which were rapidly becoming too fragile for this purpose.

From the above table it is seen that the strips of onion-scale membrane showed a decrease in numerical value from 77 to 30 in 6 hours and a complete loss of coherence in 12 hours. The onion-leaf tissue showed a decrease in numerical value from 363 to 210 in 6 hours and to 200 in 12 hours. The potato-tuber discs showed a decrease in numerical value from 127 to 27 in 6 hours and a complete loss in 12 hours. The checks apparently remained unchanged. These data are shown graphically in Fig. 6 (p. 386). The curves appear typical for enzyms. In addition to data secured on the strips of green-onion-leaf tissue it was noted that a marked loss of color took place in from 30 to 60 minutes after exposure to the action of the extract. This change in color appeared to be due to the action of the extract on the protoplast. It should be noted that because of the presence of the epidermis the extract could attack these pieces of tissue readily on but one side.

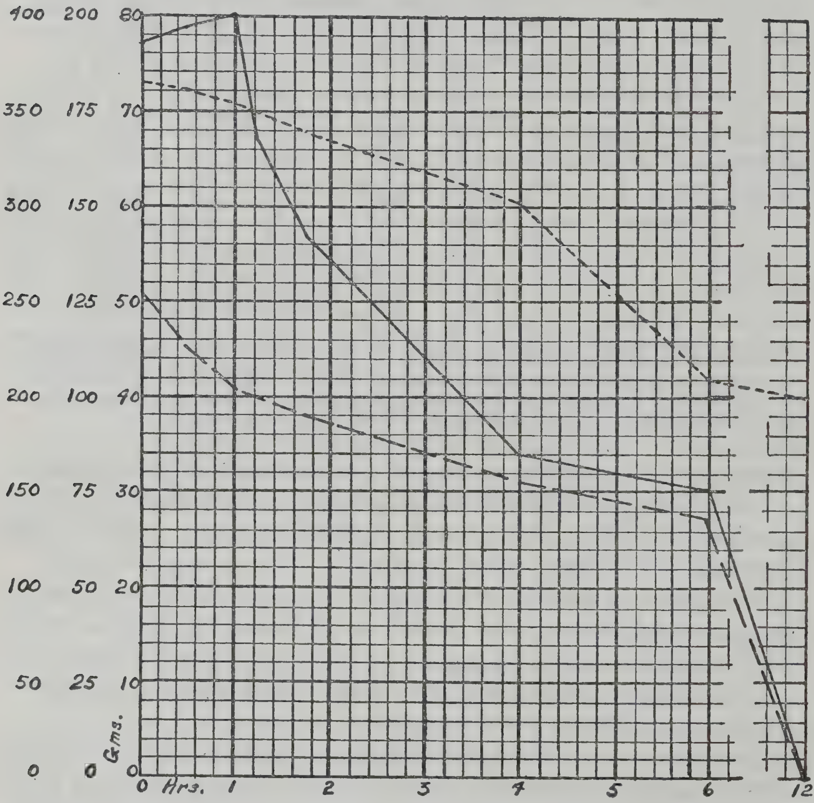

FIG. 6.— CURVES SHOWING ACTION OF ENZYM (FUNGAL EXTRACT) ON PLANT TISSUES, (————) ONION-SCALE MEMBRANE, (- - - - - -) ONION-LEAF TISSUE, (— — — —) POTATO-TUBER DISCS.

The data from these tests, together with the evidence secured by examinations of the tissue with the microscope, seem to yield but one conclusion, namely: That the action of the extract is of a two-fold nature, — (1) solution of the middle lamella resulting in loss of coherence followed by (2) disintegration of the remainder of the cell. Solution of the middle lamella allows the tissue to fall apart readily and the subsequent disintegration of the cell changes the tissues to a structureless mush. The final stage, however, does not appear to be brought about as readily as is the preceding one. The action of the extract upon the tissue was in all cases quite similar to that induced by the action of the fungus itself upon the host tissue.

Injection of the extract.— One cc. portions of the standard fungal extract were drawn up into a sterile hypodermic syringe and then injected into firm, healthy onion bulbs, using all precautions to secure asepsis. When the bulbs were opened five days later it was found that the tissue along the scales, for some millimeters, showed a brownish, water-soaked condition. Examination of the discolored tissue under the microscope, and comparison with apparently unchanged tissue from the same bulb, showed the cells to be loosened along the line of the middle lamella and badly plasmolyzed. The cells came apart very easily.

Action of the extract upon cotton fibers:— Five cc. portions of the fungal extract were placed in sterile test tubes. Cotton fibers or threads, also small strips of Schleicher and Schull's filter paper were then added to the extract. Observations were made from time to time over a period of forty-eight hours when the experiment was discarded. Altho there appeared to be a weakening of the tissue the results are too indefinite to permit any conclusions to be drawn.

A study of some of the physical and chemical properties of the extract. Effect of temperature upon the activity of the extract.— Quantities of the fungal extract were heated to the boiling point, then cooled and their action upon onion and potato tissue compared with that of the unboiled extract. It was found, as previously noted, that boiling completely destroys both the macerating and killing action of the extract. The action of the extract upon the tissues was practically inhibited by heating to 65° C.

Effect of age upon the action of the extract.— The dry combined sand and fungal elements composing the fungal powder were permitted to stand for over two months in a glass bottle closed with a ground-glass stopper. At the end of this time the powder was extracted in water and its action upon the plant tissues used was equally as effective as the fresh fungal extract. This is to be expected in view of the statements of Michaelis (1910). Jones (1909, p. 327) and Spieckermann (1902, p. 166), studying the enzyms produced by phyto-pathogenic bacteria, report that they were unable to note any loss in enzymic activity in dried, enzym-containing precipitate kept for months or even years. Dox (1910, p. 391) stated that the enzym powder, if stored in a glass-stoppered bottle and kept from the light will retain its activity indefinitely.

Titration of the extract.— When five cc. portions of the standard fungal extract were added to 45 cc. of distilled water and titrated with N/20 NaOH, using phenolpthalein as an indicator, it required an average of 1.1 cc. of the N/20 alkali to neutralize the acid in the 5 cc. Therefore the extract was +11° acid in terms of Fuller's scale or 1 per ct. normal acid to phenolpthalein.

Relation of chemical reaction to activity.—Brown (1915, p. 338–341), in his work with fungal extract of *Botrytis cinerea*, has shown that when the acidity of the extract was diminished by adding N/1 NaOH "the activity remained unaltered up to a certain point, when it falls sharply to zero." This author also found that in all cases when the acidity of the extract was increased by the addition of an organic or a mineral acid up to a certain point, the action on the activity of the fungal extract was one of retardation. In an alkaline solution the extract had no activity.

Jones (1909, p. 329) found that 2 per ct. alkali slightly inhibited, while 10 per ct. totally inhibited the action of the enzym pectinase of *Bacillus carotovorus*. In the case of acids a reaction of 1 per ct. normal acid and above inhibited the action, while 5 per ct. to 10 per ct. totally inhibited the action of this enzym.

Experiments bearing on this point were carried out by the writer. They are interesting and significant. The results of a single experiment will suffice to illustrate the method and the results. A quantity of the fungal extract was prepared by the usual methods as given above. Ten cc. portions of the extract were placed in each of four sterile beakers. One lot was left untreated. One lot was neutralized by adding 0.1 cc. of N/1 NaOH since it was found that with this particular lot of extract each 5 cc. portion in 45 cc. of distilled water required 1.1 cc. of N/20 NaOH to neutralize the acid, using phenolpthalein as an indicator. One lot was made alkaline by adding 0.1 cc. of N/1 NaOH to neutralize it, then adding an additional 0.1 cc. making the extract —11° Fuller's scale. When the alkali was added the color of the extract changed from the characteristic pale straw color to a clearer yellow with a faint tint of amber. One lot was made acid by adding two drops (about 0.1 cc.) of N/1 HCl thereby making the extract about +20° acid Fuller's scale. Checks were run by using distilled water in place of the extract. Strips of the thin membrane from between the onion scales and potato tuber discs cut with a microtome were then added to each of the lots of extract and the checks. The following table shows the results secured:

TABLE V.— RELATION OF REACTION OF EXTRACT TO ITS ACTIVITY.

REACTION OF EXTRACT.	TIME IN HOURS.					
	1 hour.	2 hours.	3 hours.	4 hours.	5 hours.	6 hours.
Untreated........	Unchanged.	Slightly softened.	Slightly softened.	Becoming very soft.	Coherence almost lost.	Total loss of coherence.
Neutral..........	Unchanged.	Slightly softened.	Slightly softened.	Slightly softer.	Softened considerably.	Almost as soft as the untreated lot.
Acid.............	Unchanged.	Unchanged.	Very slightly softened.	Slightly softened.	Slightly softened.	Slightly softened.
Alkaline..........	Unchanged.	Unchanged.	Unchanged.	Unchanged.	Unchanged.	Unchanged.
Checks..........	....	....	....	....	....	....

The results of this and similar experiments agree quite closely with those secured by Brown. Plainly, there appeared to be but little difference between the action of the neutralized and the untreated extract. No change took place in the tissues in the alkaline solution. At certain points of acidity the action of the acid extract was retarded quite markedly. The action of the extract upon the onion tissue, in all cases where it could be detected, was quite similar to the action of the fungus itself upon the onion bulbs.

An attempt to secure and purify the enzym by precipitation with alcohol.— All of the preceding experiments seem to give conclusive evidence that an enzym is produced by the onion Botrytis which has a pronounced effect upon the tissue of the host. The following studies were made in an attempt to secure the enzym by precipitation with alcohol.

Methods.— A quantity of the fungal extract was prepared by the methods previously given. This was carefully measured and enough 95 per ct. alcohol added to make the solution 80 per ct. alcoholic, since Jones (1909) has shown that with the enzym pectinase the best results were secured with this concentration. Immediately after the alcohol was added, a medium to heavy, white, flocculent precipitate was formed thruout the liquid. In the course of a few hours, this precipitate had gradually settled to the bottom of the graduate. The clear liquid was then siphoned off, and the remainder poured upon a sheet of high grade filter paper. The liquid passed thru readily leaving the precipitate on the filter paper where it was washed with absolute alcohol. The filter paper containing the precipitate was then held for a few minutes in a current of dry, warm air from an electric oven. When partially dry the paper and precipitate were lowered into a desiccator over sulphuric acid. After remaining in the desiccator over night, the gray precipitate was scraped from the paper and powdered. The powder was then redissolved in sterile distilled water, using 50 cc. to about 0.2 gram of the precipitate. After standing for four hours with occasional shaking the powdered precipitate appeared to be entirely dissolved. Discs of onion-bulb and potato-tuber tissue were then added to the solution and it was found that in the course of four hours softening of the tissues had taken place. The softening continued until the pieces of tissue lost their coherence. When viewed under the microscope the cells were separated, their contents plasmolyzed and granular. The cell walls were wrinkled and folded. This experiment was repeated with the same results.

Effect of temperature upon the dissolved precipitate.— Tests were made of the action of boiled and unboiled dissolved precipitate upon tissues. The boiled solution showed no noticeable action upon the tissue discs, while the unboiled solution showed pathological changes as noted in the preceding paragraph.

Reprecipitation of the enzym.— After using the dissolved precipitate solution for some of the tests noted above, the tissue discs were removed and the solution made 80 per ct. alcoholic when a white, flocculent precipitate was secured quite similar to that observed when the fresh fungal extract was made alcoholic. To further test the action of the reprecipitated enzym, a sufficient volume of the fungal extract was secured. This was made 80 per ct. alcoholic and the usual precipitate secured in due time. The precipitate was dried, powdered, and redissolved in water. This process was repeated three times to the point where the powdered precipitate was redissolved in water, when tissue discs were added. The action of this triple-reprecipitated and dissolved precipitate upon the tissues exposed was apparently the same as the former solution tested except than its action was somewhat retarded. In other words, it retained its active principle thruout this treatment, without appreciable loss of cytolytic action thru precipitation or re-solution. No diminution in the amount of precipitate was apparent.

Precipitation of the enzym from old mycelium.— The mycelial crusts, or wefts of fungous growth upon flasks of prune juice, were lifted off with a sterile rod and forceps at the end of 45 days and placed in a sterile mortar. A small quantity of sterile, clean sand was sprinkled over the mass, which was then ground to a pulp. Enough sterile distilled water was added to give it fluidity when it was poured upon a filter consisting of high-quality absorbent cotton placed over sterile cheesecloth. This was then squeezed. The liquid which passed thru was of a straw color and had a sweet, sickening odor. Fifteen cc. portions of this were then placed in graduates and 80 cc. of 95 per ct. alcohol added to each. Just as soon as the alcohol was added a thick, stringy, rubbery precipitate, having a dirty white color was formed. If left undisturbed this precipitate, which was thought to be of a proteinaceous nature, settled to the bottom of the graduate and took on a brownish white color. Using a glass rod this heavy precipitate was very easily collected and removed from the solution. In either case, whether removed or permitted to remain, a second white precipitate appeared in the course of approximately one-half hour's time. In the case of those lots from which the first heavy precipitate had been removed, this white, flocculent precipitate was readily filtered off by means of filter paper. This second precipitate was dried, powdered, and redissolved in water exactly as was done with the precipitate from the fresh fungal extract as given on a preceding page. When redissolved in sterile distilled water its action upon onion and potato tissue appeared to be similar in all respects to that of the precipitate from the fresh fungal extract. This precipitate was readily redissolved, reprecipitated and redissolved. Ward (1888, p. 353) appears to have secured quite similar results with his lily Botrytis. In this connection it should be noted that when a small piece of the fungal crust was lifted from the prune-juice-culture flask and floated upon the surface of an 80 per ct. alcoholic solution a fine, white precipitate was noticed in the region of the lower surface of the crust or weft. The writer is not prepared to state whether this precipitate was due to the presence of the enzym or was precipitated proteinaceous matter.

Conclusions.— The results obtained from a study of the action and properties of an extract from the old and young Botrytis mycelium, and a consideration of the possibility of oxalic acid being the toxic substance excreted, forces one to conclude: (1) That the fungus secretes a middle-lamella-dissolving enzym. At least, the active principle of the extract from the old and the young triturated mycelium is to be found in the alcoholic precipitate and responds to many of the physical tests used for the detection of enzyms. (That both the macerating and lethal actions upon the host are due to the same enzym has not been proven since the precipitate secured from the fungal extract may have consisted of two or more enzyms.) (2) That the toxicity of the extract is not due to either oxalic acid or oxalates, since tissue discs in oxalic acid solutions show much less marked effect than similar discs in the fungal extract, when length of time and pathological changes in tissue are considered.

If we accept the work of Cross and Bevan (1895), Green (1901), Allen (1901), Bigelow and others (1905) as to the composition and origin of the middle lamella, and the results of Fremy (1840, 1848) and Bourquelot and Herissey (1898) as to the enzym capable of hydrolyzing the pectic elements in the middle lamella, we must adopt the nomenclature of Jones (1909), Euler (1912) and the classification given by Haas and Hill (1913) and refer to the middle-lamella-dissolving enzym produced by this fungus as pectinase.

Action of the fungus on cellulose.— From a study of the diseased or fungus-invaded tissue of the onion bulbs it seemed evident that the cellulose walls were attacked by the fungus or its enzyms. An attempt was made to determine whether the fungus produced an enzym capable of attacking cellulose by employing the following methods: The fungus was grown upon solutions poor in organic matter with and without the addition of cellulose. The solution which gave the most satisfactory results was that employed by Reed (1912) and was made up as follows:

Ammonium nitrate	10.0 grams
Dipotassium phosphate	5.0 "
Magnesium sulphate	1.0 "
Sodium citrate	1.0 "
Distilled water	1000 cc.

Working along the line suggested by Coons (1916) filter-paper cones were placed in small culture dishes. Ten cc. portions of the solution were then added to each of the dishes containing the filter paper, and also to an equal number containing no filter paper. The two lots were then autoclaved. Inoculations were made with spores, a few dishes of each lot being left uninoculated as checks. In four weeks' time a copious growth showing abundant conidium formation, had taken place on the filter paper cones, but only a very small weft of the mycelium developed in the dishes without filter paper.

In addition to the cultures in small culture dishes, 500 cc. Erlenmeyer flasks were used. Two hundred cc. of the solution were placed in each. To one of each pair of flasks 2 grams of Schleicher and Schull's filter paper were added, both were then sterilized in the autoclave and inoculated. There was considerable mycelial growth and some spore production on the filter paper, but only a minute tuft of mycelium on the solution without the filter paper.

After 90 days' growth the solutions beneath the cultures were titrated with N/20 NaOH using phenolpthalein as an indicator. The solution to which the filter paper had been added and upon which the copious growth occurred showed a titer of +47° acid Fuller's scale. The titer of the solution to which no filter paper was added was +31° Fuller's scale. Neither the culture flasks with the filter paper nor the controls without paper showed any reducing action upon Fehling's solution, indicating that the small amounts of sugar, if formed, were probably used immediately by the fungus.

These results with the filter paper cellulose appear to be strong evidence that the fungus produces an enzym, cytase, which has the power of attacking cellulose. This may in part explain the action upon the cell walls of the host tissues as noted elsewhere. In this connection it is worthy of note that species of Botrytis have been reported as attacking cellulose by De Bary (1886), Ward (1888), Miyoshi (1894), Behrens (1898), Van Iterson (1904), and Ruhland (1908). Experiments dealing with the demonstration of cytase production by the fungus are not completed. These will be reported at a later date.

IMMUNITY RELATIONS.

Relative resistance of different varieties of onions.— It is a fact well known to onion growers that the thin-skinned white varieties suffer very severely from the neck rot. There appears to be but little difference between the relative resistance of the red and yellow globe varieties to the neck rot. However, it is believed by some growers that the red varieties are not as easily affected. The difference in these cases appears to be partly one of degree of maturity and not a varietal difference. No difference in susceptibility to neck rot could be detected between the various shapes of onion bulbs whether round, flat or globe shaped; however, it was found that the extreme globe or "bottle neck" types were slower in drying out at the neck and consequently showed a greater percentage of neck rot.

CONTROL.

FACTORS WHICH FAVOR INFECTION OR AMOUNT OF NECK ROT.

MATURITY AND DEGREE OF CURING.

The condition of the bulbs when ready to harvest has an important bearing upon the amount of neck rot which may develop in storage. Bulbs which have large green tops, and "bull-necks" are especially susceptible to neck infection. It is preferred that the tops ripen down and shrivel, and the outer skin be dry before pulling the crop. In this connection the amount of onion mildew (*Peronospora Schleideniana*) has an important bearing upon the amount of neck rot which may develop in storage. Onions attacked by the mildew fungus are as a consequence poorly matured and soft and are especially susceptible to the neck-rot disease. This fact was strongly emphasized by one prominent grower who said that if they could get their onions thru the season without being attacked by the mildew, they would not lose so heavily from the rot in storage.

FERTILIZERS.

Fertilizers, so essential in the production of a crop of onions, were found to have no noticeable relation to the amount of neck rot except in instances where applied in incorrect proportions or very late sowings were made, or where large quantities of manure have been applied to the land just previous to planting. These practices result in an over-production of tops which die late in the season affording an excellent opportunity for infection thru the dying necks. The bulbs from such plants are usually small and soft, and later show a high percentage of rot in storage.

SOIL TYPES.

Early in the study of this disease it was believed that the fungus was more prevalent in the muck soils, since muck onions showed a comparatively larger amount of rot. It was found, however, that the amount of neck rot depended largely upon other factors, mainly degree of maturity of bulbs, extent of curing and the weather at curing time, and the storage conditions under which the onions were placed. Upland onions often die and dry down earlier in the season, and the bulbs are often firmer as a result. A number of instances have been noted where red onions growing upon upland of a loamy character have shown considerable neck rot in storage. One instance came to the writer's attention where the sclerotia of the fungus were found upon the bulbs grown upon a loamy soil, at the time of placing them in the storage house in early fall. None of the onion growing regions visited by the writer were found to be entirely free from the neck-rot disease.

AIR DRAINAGE.

Air drainage of the onion fields appears to be an important factor. One small onion-growing section was studied in particular since it had the reputation of consistently yielding onions which later developed considerable neck rot in storage. The factors at work here were plainly the high hills and dense woodland on two sides of the onion fields. The dews and rains did not evaporate readily and the crop remained wet during the greater part of the forenoon. This is especially detrimental during the harvesting and curing periods.

STORAGE CONDITIONS.

Careful investigations have shown that of all the various factors considered which have a bearing upon the development of the neck-rot disease, the control of storage conditions is the most important.

The factors which favor onion neck rot are overheating and poor ventilation in storage houses. It must be admitted, however, that the very best houses cannot carry a crop safely thru the storage season when the bulbs are soft and poorly cured, and moist weather prevails soon after storage begins. A considerable number of the houses used for onion storage are entirely unsuited for this purpose. One particular storage house, having a capacity of approximately 20,000 crates and in which several thousand crates were lost thru neck rot in 1915, was visited by the writer for the purpose of securing some data as to the relative humidity of the house. This house was of concrete construction. It had a rather low, flat roof, only three or four doors along each side of the building and very meager provisions for ventilation thru the roof. The house has single walls and is heated by steam, the pipes running along the outside walls. As cold weather came on and steam was passed thru the heating system it vaporized all the frost on the walls and pipes, the moisture passing thruout the stored onions bringing on a considerable amount of neck rot in the course of a few days.

Ventilation is of prime importance. The roof should be of a steep pitch, and preferably double, to draw out the moist air. The proper regulation of the ventilation vents is important. These should be closed during damp, foggy, misty weather. The writer traced a severe epidemic of neck rot in one storage house to the fact that the owner opened the doors and left them open during a warm, misty day in mid-winter while a carload of onions was being sacked and removed from the storage house.

Records of the temperature and humidity in onion storage houses were obtained by the use of carefully adjusted Lambrecht polymeters. These records show that a relative humidity of 65–70 per ct. coupled with a temperature of 16–22° C. is very detrimental to onions in storage.

A study of the temperature relations of the fungus show that the temperature of the storage house must be kept very close to 0° C., or about 34° F., in order to prevent the development of the neck-rot fungus. Ames (1915) has shown that a temperature of near zero Centigrade (32° F.) must be maintained to prevent the development of rot fungi in refrigerating experiments.

The onion storage house at South Lima, New York, operated by Mr. C. C. Mowris, was placed at the writer's disposal for the purpose of storage observations. Very little neck rot occurred here. This house, which is one of the best, shows a very desirable type of construction providing ample means for ventilation and heating (Plate XXIX, fig. 2).

STORAGE EXPERIMENTS.

Occasionally one finds notices posted on onion warehouses to the effect that " no onions having necks or stems longer than one and one-half or two inches will be accepted." Because of the fact that some onion buyers lay particular stress on the length of neck of onions for storage, claiming that long-necked onions do not keep as well as the short-necked ones, some tests were made upon this point. On September 24, 1913, the writer visited a field in which some neck rot had occurred the previous season, and also during the present season, in crates of onions then in windrows. Six crates of these onions were prepared for an experiment. In two the bulbs were topped very short; in two they had necks 3–4 inches long; while in the remaining two the tops were left uncut. Three of the crates (one of each pair) were stored in a very dry, cool place (the loft of a large building). The other three were stored in the rather damp basement of a storage house where the ventilation was very inadequate tho similar to that of some onion storage houses. These two lots of onions were kept in these storage places until March 20, 1914, and data secured from time to time as to the number of bulbs affected with neck rot. A summary of the data shows that an average of approximately 45 per ct. of the bulbs in the damp storage were destroyed by the neck-rot fungus and the remainder sprouted. Of the bulbs in the very dry storage between 2 and 3 per ct. were destroyed by the neck-rot fungus while only a few bulbs sprouted. As to the effect of short versus long topping and tops uncut upon the amount of neck rot no data could be secured since all were rotted alike in the damp storage. In these experiments the length of neck did not appear to be an important factor, the humidity of the storage place being the factor which influenced keeping quality.

A similar experiment with mildewed onions was carried along with the above-mentioned series and as with the normal crop onions the length of neck proved unimportant; the humidity of the storage quarters determined the keeping quality. The mildewed onions showed neck rot quicker in the damp storage than did the normal crop. It was evident that the mildewed crop was not as mature and carried more moisture into the storage with it.

The results of these experiments indicate that the length of neck, whether one or three inches long, has no relation to the amount of neck rot.

In an attempt to determine how soon after being placed in storage the neck rot would appear upon the onion bulbs, an experiment was conducted in which thoroly ripe and immature bulbs were compared. On September 3, 1913, crates of ripe and of immature onions were taken from windrows in an onion field in Livingston County, New York, and immediately transported to an onion storage-house in which considerable neck rot had occurred the previous season. On September 24, or 21 days later, the neck-rot fungus could be found fruiting on a considerable number (about 10 per ct.) of the bulbs in this rather damp storage house even tho the bulbs were in new crates not used for onions before. The immature bulbs showed a greater development of the disease.

These and similar storage experiments emphasize two important points; namely, that the crop must be dry and mature and stored in a dry, well ventilated house in order to materially check the development of the neck-rot fungus.

STUDY OF REMEDIAL MEASURES.

SPRAYING.

As to the effect of spraying onions with bordeaux mixture upon the amount of neck rot which developed in storage the writer has the following data to offer. Thru the courtesy and cooperation of Mr. Henry Greffrath of South Lima, N. Y., the crops from two fields of onions, one sprayed with bordeaux mixture, the other unsprayed, were placed in storage and when the stock was sold at the end of the storage period observations were made on the amount of shrinkage, due mainly to neck rot and sprouting. The data are as follows: 2315 bushels unsprayed onions sold March 1, 1914, gave 15.2 per ct. shrinkage; 942 bushels sprayed onions sold April 8, 1914, gave 10.8 per ct. shrinkage, a gain of 4.4 per ct. due to spraying three times with 5–5–50 bordeaux mixture. It is to be noted, however, that the sprayed onions were removed from the storage over one month later than the unsprayed crop. It is also worthy of note that the sprayed stock was topped by a topping machine while most of the unsprayed stock was hand topped. Attention has already been called to the fact that many onion growers claim that hand-topped onions will keep better than machine-topped stock. It may be well to state that both lots of onions were stored in the same storage house and under the same conditions and that the storage was slightly overloaded which is very unfavorable to long keeping.

The spraying outfit used by Mr. Greffrath was one designed by himself for the purpose of spraying celery and onions. It consisted of a boom carrying fourteen nozzles eighteen inches apart, attached to a field power sprayer by two leads of hose. The sprayer is driven along the alleys, which usually consist of two rows of celery or lettuce between wide strips of onions, and the boom is carried by two men over the onion rows. No resin sticker was used in the bordeaux mixture since it was found that by going over the rows twice, i. e., once each way after short intervals of time, carrying the boom with the nozzles pointed slightly forward, and using high pressure (200 lbs.), the onion foliage could be completely covered by the fine, mist-like spray.

In a series of smaller experiments conducted at the Geneva Station, in which no data as to yield were secured, it was found that for a combination spray for onion mildew, neck rot and thrips, 5–5–50 bordeaux mixture to which were added Black Leaf 40 at the rate of three-fourths pint to 100 gallons of the spray mixture, and soap at the rate of 5 pounds per 100 gallons could be used with comparative safety on onions. A very slight discoloration of the leaves occurred where the mixture collected in drops. The nicotine extract in the mixture appeared to be quite effective against the thrips.

There are several serious difficulties met with in the spraying of onions which tend to make onion spraying very unpopular among onion growers. The most important of these difficulties is the tendency of the tops of the plants to drop over and become entangled just at the period when fungus diseases appear, making it very difficult to pass thru the field. Also on account of the glaucous character of the onion leaves, it is very difficult to make the spray stick to the foliage when using one of the low-pressure outfits even when the resin sticker is added.

Clinton (1904) reported some spraying experiments designed to determine the value of bordeaux mixture as a preventive of neck rot on white onions. Three applications of the spray mixture were made, but since no neck rot developed in storage no data were obtained upon the relative keeping qualities of the sprayed and unsprayed stock.

FUMIGATION.

The fumigation of onions with formaldehyde gas, either just before or just after they are placed in storage houses, has been recommended by Selby (1910) and Humbert (1916) as an efficient method of preventing the neck-rot disease. In order to test the efficiency of the gas against the neck-rot disease, quantities of onions, varying from a few specimens to one bushel lots were subjected to the treatment for twenty-four hours. The fumigation chamber used was a wooden box of 21.56 cubic feet capacity constructed of matched lumber and painted on the inside. The fittings of the door, which was on one side, were perfectly tight and but very little odor of escaping gas could be detected during the experiments. The gas was generated by the permanganate method, using 30 cc. of formaldehyde solution and 14.25 grams of potassium permanganate for the 21.56 cubic feet of space. These quantities are equivalent to 23 ounces of permanganate and 3 pints of formaldehyde per 1000 cubic feet of space as recommended by Morse (1907) for the disinfection of potatoes. The details of all of the seven tests with gas will not be given. The results of one or two typical tests will suffice to illustrate the results secured. On October 2, 1913, a Georgia peach basket, containing about forty onions of both red and yellow globe varieties secured directly from the field where they were cured and made ready for the storage, was placed in the fumigation chamber. The charge of chemicals was prepared, mixed and the door closed immediately. The charge of gas was permitted to remain in the chamber for twenty-four hours when the door was opened and the basket of onions removed. Upon examination it was found that the gas had injured the onions where the outer dry skins had peeled off exposing the flesh of the scales. The injury was in the form of sunken areas or pits (Plate VIII, fig. 3) and in some cases appeared as distinct blisters. During the experiment the fumigation chamber stood in a storage barn where the conditions were practically identical with those found in an onion storage house. After an examination was made the basket of onions was immediately taken to a potato storage cellar where it remained thirty-nine days. At the end of this period it was found that one bulb showed the typical black sclerotia at the neck end. The fungus mycelium must have been present in the bulb during fumigation. Cultures confirmed the macroscopical observation. Four out of six cultures, made from different places among the onions, showed that, in this case at least, the gas was not effective against the fungus. This lot of onions was kept in storage until April 2, 1914, when they were discarded. Slightly over 10 per ct. of the bulbs showed the neck rot at that time. In this connection the experiments of Massee (1913) are of interest. This investigator experimented with the Botrytis causing the lily disease and found that pieces of lily stem containing the hibernating mycelium, even when soaked for twenty-four hours in hydrogen peroxid and later cultured, showed abundant growth and fruiting of the fungus.

In another experiment twelve apparently sound onions were placed in the fumigation chamber and treated for twenty-four hours with twice the amount prescribed in the Maine formula. After twenty-four hours the injured areas on the bulbs showed as raised blisters and the scales had a greenish tint. After being in storage forty days one of the twelve bulbs showed the neck-rot fungus breaking thru the outer scales. Four months later two of the bulbs were infected, showing that double strength gas treatment was not effective in controlling the fungus.

In a third series of experiments, both single and double strength gas caused conspicuous injury to the freshly exposed scales. When using the gas treatment upon either the colored or the white varieties of onions no injury occurred to the scales which were completely enclosed by a dry skin.

On October 6, 1913, a bushel crate of yellow onions was placed in the chamber and treated with the gas for twenty-four hours, after which it was removed and placed in a potato cellar along with another crate of onions from the same lot which was not fumigated. The storage conditions were perhaps typical of the average onion storage house in New York State. These were left undisturbed until April 6, 1914, or six months later, when they were removed and examined. Both the fumigated and unfumigated stock showed a large amount (about 65 per ct.) of neck rot. Many of the bulbs showed the sclerotia at both ends, and many of the bulbs had sprouted and the fungus had reduced the sprouts to a mass of soft, brownish tissue.

Conclusions.— From these and similar experiments the writer is forced to conclude that fumigation of onions with formaldehyde gas against the neck rot is ineffective, mainly because it does not reach the mycelium in the bulbs. Furthermore, the average onion storage house is not tight enough to retain the fumes. The crates of onions usually contain a considerable amount of dry skins which prevents the penetration of the gas.

METHODS OF CONTROL.

From the data and experimental evidence at hand it is evident that the control of this disease lies mainly along the line of careful cultural measures and storage house control. Since it is evident that the greater part of the neck and bulb infection takes place late in the season, especially when the crop is curing, every precaution should be exercised to have the crop mature and die early. Late maturity may be prevented by avoiding the very late application of fertilizers and manures, especially fertilizers of an incorrect formula, and also by careful selection of the seed stock, being careful to avoid the types of onions with a thick neck. Air drainage has been found to have an indirect effect on the amount of neck rot and also an important bearing upon the prevention of onion mildew. The growing of onions in fields surrounded by hills and dense woods should be avoided when possible. Removing the tall weeds along ditches, or still better, by replacing the ditches with covered tile drains has been found to materially lessen the amount of neck rot, mainly by facilitating better drying of the crop. However, it is often necessary to provide wind-breaks of arbor vitae or picket fences along one side of the field to prevent wind injury to the crop.

Care should be exercised in handling the crop since infection occurs thru bruises, shovel, fork and topper wounds. As far as possible prevent bruising the stock while running it over the topping machine. The onions should be topped fairly short — 1 to 2 inches. The removal of excess soil, screenings, and tops by screening in the field will facilitate drying in crates. After a thoro drying in the windrows the crates of onions should be kept in the cribs and drying sheds as long as possible. The bulbs should be stored in slatted crates of standard size. The use of bags or bins cannot be too strongly condemned. The full crates should be stacked with 1 x 3 inch binders between them, to facilitate ventilation. Onions stored in large, loose piles undergo a sweating process which aggravates the neck rot. The regulation of the onion storage house is most important. The neck rot cannot be controlled in storage unless good air circulation is provided by means of ventilators at top and bottom which can be opened and closed at will to control the temperature. The temperature of the storage house should be carried as low as possible without freezing the bulbs. A temperature just above 0° C., or 34°–36° F., is desirable. After the onions are thoroly chilled the storage should be kept closed to hold the temperature down. The storage house should not be opened for any considerable length of time especially during warm, damp, rainy or foggy days. If this is done the damp outside air will rush in and the entire stock will become covered with moisture and severe bulb rotting will follow. A relative humidity of 65–70° is very detrimental to keeping quality. Artificial heat, when needed, can best be supplied by stoves, or, at least, by dry heat. Steam heating is conducive to considerable rot since the steam pipes when not in use in cold weather become covered with frost and then, if steam is passed thru them, the humidity of the house is raised to a high degree.

Storage houses with a low, flat roof and poor top and bottom ventilation are to be avoided for onion storage. They should be constructed double thruout, using a good quality of lining for the walls. Concrete houses must have double walls with a dead-air space and must be sheeted up on the inside. The roof should have a steep pitch with large ventilators at the extreme top. The floors should be of heavy material with spaces of about one-half inch between each narrow piece of flooring. This is especially essential when onions are stored below the first floor.

The old, rotting onions should not be used as a fertilizer on the onion fields since it has been proved that they are a means of carrying the fungus to the fields and to the growing crop thru the mycelium and the sclerotia. Piles of decaying bulbs should not be permitted to remain in the vicinity of onion seed fields.

Especial attention should be given to the selection of sound, healthy seed or " mother bulbs " for the seed crop, since a few diseased bulbs may serve as a dangerous source of infection for the entire seed crop if the season is a wet one.

The treatment of the seed used for sowing the market crop by soaking it for 20 to 30 minutes in a solution made by adding 1 ounce of formalin to 2 to 3 gallons of water is being used by a number of onion growers. This is used as a preventive of onion smut and also as a precautionary measure against onion blight. Altho spores of the neck-rot fungus have been found on the seed, the possibilities of seed treatment for this disease have not been fully worked out experimentally. Seed treatment appears to be a safe precautionary measure. The light seed floating on the surface of the disinfecting solution may be skimmed off.

Spraying the crop with 5–5–50 bordeaux mixture or resin bordeaux, beginning early in July, and making 3 or 4 applications, has been found to be of some value in preventing neck rot, but extensive data on the value of spraying are not at hand. It has been practically demonstrated that the prevention of onion blight has a marked influence upon the amount of neck rot which may follow when poorly cured, soft, blighted stock is placed in storage.

Fumigation of yellow and red varieties of onions with gas either before or after the bulbs have been placed in storage cannot be recommended as a precautionary measure. In the first place onion storage houses are, as a rule, not tight enough to retain the fumes; furthermore, it has been found that the mycelium of the fungus is already within the necks of the bulbs before they go to the storage house. Any disinfectant which would kill the fungus in such cases would necessarily destroy the onions.

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REPORT

OF THE

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(Connected with Grape Culture Investigations.)

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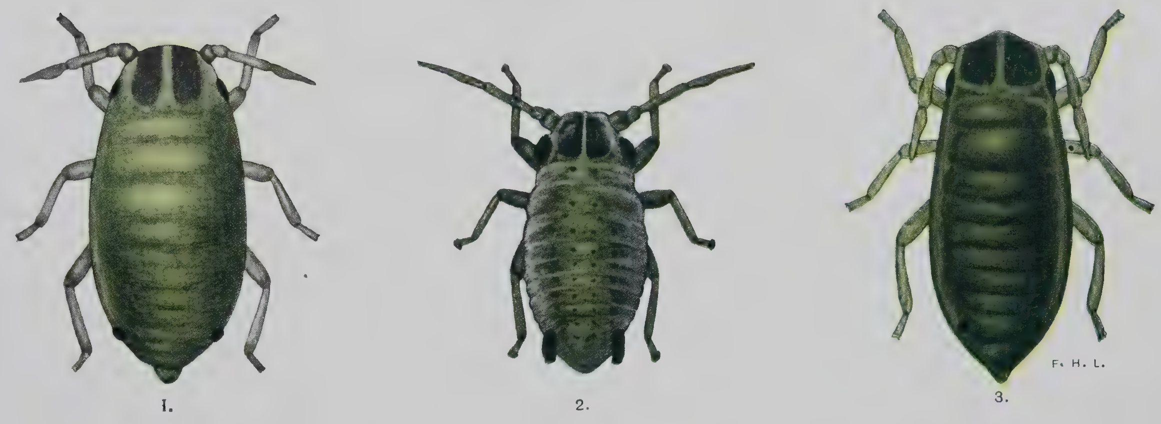

PLATE XXX.—LARVÆ OF FIRST INSTAR: 1. APHIS POMI 2. APHIS SORBI 3. APHIS AVENÆ

REPORT OF THE DEPARTMENT OF ENTOMOLOGY.

PLANT LICE INJURIOUS TO APPLE ORCHARDS. II.*

STUDIES ON CONTROL OF NEWLY-HATCHED APHIDES. II.

P. J. PARROTT, H. E. HODGKISS and F. H. LATHROP.

SUMMARY.

This is the second report of life history studies and experiments with the apple aphides (***Aphis sorbi**, avenæ* and ***pomi***), which have for their object the establishment of efficient spraying practices for the protection of bearing apple orchards.

The newly-hatched nymphs of the three species are tiny insects which are quite similar in general appearance and habits. They are readily separated by structural differences, principally of antennæ and cornicles. Descriptions are given of first instar nymphs and adults of first generation in which recognition characters are clearly indicated.

In general, infestation of orchards in western New York during 1916 was not severe. At the outset the oat aphis outnumbered the rosy aphis and the green aphis in most orchards, altho their comparative numbers varied locally. The relative abundance of the three species exhibits a seasonal succession or cycle which, while probably varying somewhat from year to year, appears to be fundamentally constant. At Geneva activities began with the hatching of goodly numbers of the oat aphis on April 22. A few rosy aphides were detected on the buds at the same date, but this species did not reach maximum numbers until four days later. The green aphis was the last species to hatch and began to emerge on April 26. As during the preceding year, dwarfing of apples was chiefly the work of the rosy aphis; altho losses in fruit yields in this respect were rarely of serious extent.

In experiments with the aphides all species attacked succulent tissues, as blossom and fruit stems, tender leaves and young apples.

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* Reprint of Bulletin No. 431, March, 1917.

As a result of their activities various distortions of apples developed. Severe infestation by *A. sorbi* and *A. pomi* was attended in a number of instances by destruction of entire clusters of apples.

In an experiment on Rome apples an application on May 1 of lime-sulphur and nicotine sulphate at recommended strengths afforded efficient protection from the oat aphis and the rosy aphis. The trees receiving the treatment were also free from the green aphis until June, when there was a re-infestation of the plat due to invasion by winged migrants.

Of twelve auxiliary experiments, nine gave appreciable benefits from spraying. The remainder were inconclusive because of slight infestation by the insects or excessive defoliation by apple scab assisted in part by aphides.

As losses by aphides vary according to locality and seasonal conditions, the experiences of orchardists are the best criteria of the necessity of systematic spraying to combat the insects. To obtain positive evidence of the destructive influences of aphides on the apple crop and the effectiveness of spraying, growers should conduct a test, for which directions are given. The results of such an experiment continued for a period of years ought to indicate the practicability of undertaking larger operations as a regular procedure in the upkeep of apple plantings.

INTRODUCTION.

In continuance of the plan previously outlined in Bulletin 415 of this Station, various studies on the life histories and habits of several species of apple aphides and experiments to develop more efficient spraying practices were repeated during the past season. The more important results and applications from these efforts are considered under the following captions:

1. Classification of newly-hatched larvæ and stem-mothers of first brood.
2. Seasonal behavior of aphides.
3. Influence of aphides on fruits.
4. Test with lime-sulphur and nicotine solution.
5. Auxiliary experiments.
6. The delayed dormant application in the spraying schedule.
7. A plan for a spraying test.

CLASSIFICATION OF NEWLY-HATCHED LARVÆ AND STEM-MOTHERS OF FIRST BROOD.

The newly-hatched nymphs of the three common apple aphides are tiny insects closely similar in appearance and in habits. All are usually of a dark green color, and it is only by close examination that the several species may be distinguished. While the adults are much more easily separated, it is frequently important that the species be determined at the very beginning of the season, before maturity is reached. These stages of the different species may be distinguished by the accompanying descriptions.

IDENTIFICATION OF NEWLY-HATCHED NYMPHS.

Aphis sorbi Kalt. is of a very dark green color, in the first stage, with a pair of darker areas on the head and several rows of dark tuberculate spots extending lengthwise of the body. The antennæ, legs and cornicles or " honey-tubes " are dark in color. The antennæ are long and slender, reaching nearly to the bases of the cornicles, which are long and prominent, somewhat enlarged distally and flanged at the tip. The entire insect is more or less covered with a whitish powder, giving it a rather bluish appearance, which, together with the longer antennæ and cornicles, at once distinguishes this species from both *pomi* and *avenæ*. (Plate XXX, fig. 2, and Fig. 7, b.)

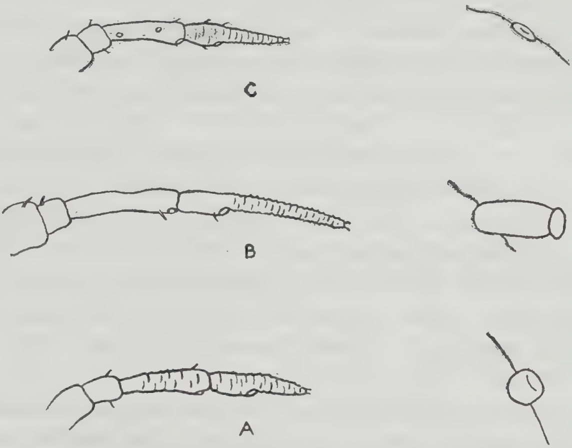


Fig. 7.—Antennæ and Cornicles: a, *pomi;* b, *sorbi;* c, *avenæ.*

Aphis pomi DeGeer is usually of a dark green color, a trifle lighter than the corresponding stages of *sorbi* and *avenæ*, varying, however, to almost a lemon yellow. The head is dusky and rather brownish. The antennæ are intermediate in length between those of the other species under consideration, and are dusky in color, especially toward the apex. Legs darkened on the distal ends of the segments. Cornicles dark colored and conical in shape. The distinguishing features of this species are the moderate length of the antennæ, and the conical "honey tubes," which are shorter than those of *sorbi*, yet considerably longer than those of *avenæ*. Sometimes the body is somewhat powdery but not so much so as in the case of *sorbi*. (Plate XXX, fig. 1 and Fig. 7, a.)

Aphis avenæ Fab. is of even darker hue than either of the other species. There are two large blackish rectangles on the head, while the antennæ and legs are very dark in color, and the cornicles are black. The antennæ are shorter than those of either of the other species, reaching to the middle pair of legs, while the cornicles are extremely short, being mere discs. The short antennæ and abbreviated cornicles form ready means of distinction for this species. (Plate XXX, fig. 3 and Fig. 7, c.)

IDENTIFICATION OF MATURE STEM-MOTHERS.

Aphis sorbi Kalt.— As this species approaches maturity there is a gradual but considerable change in appearance, and the adult is no longer green but of a variable and almost indescribable tint. Usually this is a dark bluish slate color, and more or less powdery. Some specimens are paler, the color varying to a rather yellowish brown. The antennæ are slender, usually reach about to the middle of the abdomen and are black in color except on the basal portion. Cornicles long, somewhat curved, and black in color. Besides the coloration, which easily separates this species from the others, a further means of identification is the fact that the stem-mothers will usually be found securely hidden within a tightly curled leaf.

Aphis pomi DeGeer undergoes a less decided modification in coloration as maturity is reached, the adults being a bright green color. The head is usually tinged with brownish and varies to blackish; the rest of the body is bright green, varying in some individuals to a lemon yellow. Antennæ are relatively about as long as those of the preceding species, and together with the legs are of a rather dusky brownish color. The cornicles and cauda are very dark brown or black, in striking contrast with the bright green abdomen.

Aphis avenæ Fab.—This species becomes much lighter in color as growth continues. By the time the insects are half grown they are pale green in color with more or less distinct darker green markings on the body. The adult is pale yellowish green with a series of transverse spots, which together form a broad, deeply serrated band extending the full length of the abdomen. The antennæ and cornicles are relatively somewhat shorter than those of either of the other species. The species is not likely to be confused with *sorbi* because of the difference in coloration, and may be readily distinguished from *pomi* by the dark green markings present in the latter species.

SEASONAL BEHAVIOR OF APHIDES INJURIOUS TO APPLE FOLIAGE AND FRUIT.

At the beginning of the season, aphis infestation in apple orchards was not severe in western New York during 1916. At the outset, the oat aphis by far outnumbered the green aphis or the rosy aphis in most of the orchards. The relative abundance of the three species varied, however, under local conditions, and in some of the Experiment Station orchards the rosy aphis actually presented a greater number of colonies early in the season than both of the other forms combined. In this case, however, even the rosy aphis infestation was not severe.

It is interesting to note that the relative abundance of the three species passed through a seasonal succession or cycle, which, while probably varying somewhat from year to year, must remain fundamentally constant. Activities began with the hatching of the oat aphis, which was first observed at Geneva on April 22. On this date considerable numbers of this species were present on the apple buds, and the very young nymphs continued to appear for several days. Simultaneously, a few nymphs of the rosy aphis were found in company with the oat aphides, but it was not until four days after the first observation that they reached their maximum abundance. The green aphis was the last to appear, the first nymphs emerging from the eggs on April 26. Three days later, while there were still a few unhatched eggs, the great majority of the nymphs had emerged and were feeding on the green buds. It was observed that there was a characteristic difference in the distribution of the eggs of the green aphis as compared with those of the other two species. That is, the eggs of the green aphis, while not occurring in numbers thruout the orchards generally, were localized, and occasional twigs could be found which were heavily infested with the eggs. On the other hand, the eggs of the oat aphis and the rosy aphis were uniformly scattered thru most of the orchards observed and, while infestation was more severe in the case of isolated and uncared-for trees, there was apparently much less tendency for the eggs to be aggregated in small, dense colonies. As would be supposed, this had considerable effect on the relative local abundance, and distribution of the several species during the early part of the season. The immediate result of this difference in egg distribution was that the oat aphis nymphs, the most common species, were to be found thruout the orchards, and there was a sprinkling of the rosy aphis nymphs in company with this species, while the green aphis was very largely limited to local colonies in which infestation was very severe. The oat aphis was the first to mature, and the rapid reproduction of this species soon greatly increased its relative abundance so that it became by far the most numerous species. Two days after the maturing of the stem-mothers of the oat aphis, the rosy aphis began to give birth to living young, and, while the rate of reproduction was fully equal to that of the former species, the initial abundance of the oat aphis kept the latter ahead in point of numbers. The green aphis was one day behind the rosy aphis in maturing, and the offspring served to increase the severity of the local centers of infestation. The second generation of the oat aphis was composed entirely of winged migrants, which began to leave the trees during the latter part of May, or at a time when the fruit had reached the size of marbles. By the middle of June this species had become greatly reduced in numbers and by the end of the month it had entirely disappeared from the apple. While the oat aphis was becoming less abundant because of migration to the summer food plants, reproduction by the second generation of the rosy aphis greatly increased the numbers of this species which was rapidly superseding the oat aphis, so that during the interval extending from about the middle to the last of June the rosy aphis was the more abundant of the two species in the orchards about Geneva. After July first migration of the rosy aphis was rapid and the numbers diminished until the latter part of the month, when the apple had been entirely vacated. The second generation of the green aphis was to a very large extent composed of winged forms, and during the last of May there was a general migration or scattering of this species thruout the orchards and numerous rapidly growing colonies were established on the tender terminal growth. The colonies of rosy aphis, which, up to this time, had, on the whole, been quite free from other forms, now began to be mixed with the green aphides. As this species increased in numbers the rosy aphis decreased, so that by the end of July the green aphis was quite abundant and was the only one of the three forms remaining on the apple; this condition continuing thruout the rest of the season.

The oat aphis.— This was the first species to appear on the apple buds at Geneva, and, owing to the late spring, hatching did not occur until April 22 or nearly a week later than the record of the preceding season. At the time of the first appearance of the nymphs most of the buds had barely begun to show green at the tips. A very few were slightly more advanced, while many of the more backward still showed no signs of life whatever. Within four or five days after the nymphs first appeared, all of the eggs had apparently hatched, and altho infestation was quite general no case of extremely heavy infestation was observed, the numbers of aphides on infested buds varying from one to fifteen in most cases, and probably averaging four or five. The stem-mothers of this species matured just as the blossom buds were beginning to show pink, the first being observed producing young on May 10, and at this time the leaves were well out and the fruit buds were fairly well separated in the clusters. As reproduction progressed the numbers of these aphides greatly increased, and the fruit clusters became literally coated with the insects. Strangely enough, there was little apparent effect on the tissues as the result of the feeding of these colonies of insects; at most, there was only a gentle curling of the heavily infested foliage. The second generation of this season consisted entirely of winged forms, which began leaving the apple as the petals were dropping, and from this time until the middle of June the numbers of this species on apple decreased rapidly. By the time the fruit had reached the size of marbles the aphides had entirely disappeared.

The rosy aphis.— The earliest nymphs of this species were observed coincidentally with those of the oat aphis, but the hatching of this species was prolonged over a somewhat greater period than that of the latter form, and young nymphs continued to appear as late as April 29. As soon as the young emerge from the eggs they migrate to the opening buds, and, like the other common species, creep down among the tender new leaves as they unfold. There is, however, apparently a more decided tendency with this species to remain with the leaves which they first attack, and not to advance to the tips of the more succulent growing shoots of the leaf clusters as seems to be characteristic of the other species. The attacks of this species result in a severe and characteristic curling of the leaves, which was first observed shortly before the maturing of the stem-mothers, and which became common with the maturing of these insects; a single stem-mother was sufficient to curl a leaf tightly. About the time the fruit buds had turned pink the stem-mothers began to give birth to the nymphs of the second generation. These nymphs showed little inclination to migrate and, as a rule, remained within the curled leaf occupied by the stem-mother until the leaf became overcrowded with the developing aphides. During the latter part of May and early June many new colonies were formed by these nymphs that were thus crowded out of the original colonies and forced to migrate to the tender foliage. The period of the dropping of the petals was marked by a great increase in the numbers of this species, due to the maturing of the second generation. Few, if any, of these developed wings. With the development of the third generation, however, the winged forms appeared, and by the middle of June the migrants were observed producing nymphs on plantains. After this period the numbers on apple began to diminish and after July first the decrease was rapid, due to the increasing activities of the natural enemies as well as to the flight of the winged forms. By the latter part of July the apple was entirely free of the insects.

The green apple aphis.— The hatching of this species at Geneva began on April 26 and was completed by May 2. The stem-mothers began to mature on May 13, as the blossoms were showing pink, and the infestation grew more severe. By far the greater part of the second generation was composed of winged forms, and during the last of May and the first few days of June there was a definite migration flight from the densely infested colonies to the uninfested parts of the orchards. Altho winged forms were produced thruout the rest of the summer, they were never sufficiently numerous to cause a definite, general migration. The effect of species on the foliage is intermediate between that of the other two species. During the early spring, curling of the leaves was not greatly in evidence except in cases of very severe infestation. As the summer broods became abundant, however, curling of the leaves became characteristic of the infested orchards.

INFLUENCE OF APHIDES ON FRUIT.

In order to ascertain more definitely the nature and extent of injuries sustained by apple fruits as a result of attacks by the different species of aphides, some studies were carried on along lines somewhat similar to the efforts of the summer of 1915.

For this work a Rome apple tree was selected which was practically free from infestation by these insects and as far as external conditions indicated, was representative of this variety in this orchard. At intervals during the spring varying numbers of aphides were placed upon apparently normal fruit clusters. After this operation each of these artificially infested clusters was immediately enclosed in a very thin, black, silk-netting bag, which was allowed to remain until the insects left the fruits or until the end of the growing season in case of *A. pomi.*

The fruit clusters may be grouped in four series, viz.: Series I, artificially infested with *A. sorbi;* Series II, infested with *A. avenæ;* Series III, infested with *A. pomi;* and Series IV, checks, which were free of aphides and covered with bags similar to those of the foregoing series.

Because of the fact that the fruit clusters were enclosed in cloth bags, it was considered necessary to pollinize the flowers artificially. On May 23 the blossoms were found to be in an ideal condition for fertilization; and so the bags were removed from the clusters and pollen was placed upon the pistils by stroking the flowers with others taken from adjoining trees. The bags were at once replaced in their former positions. At frequent intervals during the summer the condition of the fruit clusters with respect to the activities of the insects was noted and the fruits were measured. The principal details of the experimental activities with the different series are briefly summarized, viz.,

Series I. In this series are included twenty-two fruit clusters which were artificially infested with *sorbi,* as follows:

One cluster with five stem-mothers on May 8.
One cluster with twenty-five stem-mothers, May 13.
Three clusters, each with ten stem-mothers, May 15.
One cluster with eight stem-mothers, May 15.

Three clusters, each with one stem-mother, May 16.
Three clusters, each with one stem-mother, May 19.
Five clusters, each with one wingless female of the second generation, June 13.
Two clusters, each with one colony, June 21.
Two clusters, each with one colony, June 22.
One cluster with one colony, June 29.

The fruits of this series were calibrated on June 27; July 5, 19, 29; Aug. 7, 11, 18, 25, and September 1, 7, 16 and 30. The records are shown in Table I and are summarized in Table V.

Series II. This series includes nine clusters which were artificially infested with *avenæ* as follows:

One cluster with fifteen stem-mothers, May 9.
One cluster with sixty stem-mothers, May 10.
One cluster with forty-two stem-mothers, May 12.
One cluster with thirty stem-mothers, May 12.
Five clusters, each with thirty stem-mothers, May 13.

Measurements of the fruits were taken on June 27; July 5, 19, 29; August 7, 11, 18 and September 1, 7, 16 and 30. The records are compiled and summarized in Tables II and VI.

Series III. Includes seven clusters artificially infested with *pomi* as follows:

Six clusters, each with from forty to sixty stem-mothers, May 19.
One cluster with one colony, June 29.

These clusters were examined and the fruits measured on June 27, July 5, 19, 29; August 11, 18 and September 1, 7, 16 and 30. The measurements are given in Table III and are summarized in Table VII.

Series IV, checks. The ten clusters in this series were enclosed in the netting bags exactly as those of the other series, but no aphides were admitted. Two of the clusters were bagged on May 8 and the other eight clusters on May 12.

Observations and measurements were made on June 27, July 5, 19, 29, August 11, 18 and September 1, 7, 16, 30. Table IV shows the measurements, which are also summarized in Table VIII.

TABLE I.— MEASUREMENTS OF FRUITS ATTACKED BY *A. sorbi.*

No. of cluster.	NUMBER OF APHIDES.	JUNE 27.		JULY 5.		JULY 19.		JULY 29.	
		Axial.	Trans.	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	STEM-MOTHERS:	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Five, May 8.........	11.5	10.0	12.0	11.0	.....	.....	.....	......
		6.0	5.5	.....	.....	.....	.....	.....	......
2	Twenty-five, May 13..	.....	.....	.....	.....	.....	.....	.....	......
3	Ten, May 15.........	.....	.....	.....	.....	.....	.....	.....	......
4	Ten, May 15.........	10.5	9.0	.....	.....	.....	.....	.....	......
5	Ten, May 15.........	11.5	10.0	.....	.....	.....	.....	.....	......
6	Eight, May 15.......	.....	.....	.....	.....	.....	.....	.....	......
7	One, May 16.........	23.0	21.0	25.5	25.0	29.0	32.5	37.0	39.0
		24.0	21.5	28.5	26.0	34.0	37.0	34.0	41.0
		22.5	23.0	28.0	28.5	35.0	34.0	32.0	38.0
8	One, May 16.........	16.0	14.0	16.5	15.5	19.0	17.5	20.0	22.0
		14.5	12.5	14.0	15.0	23.5	22.0	23.0	20.0
		13.0	11.0	14.0	15.0	21.0	19.0	12.0	13.0
		16.5	13.0	18.0	16.5	17.5	14.5	12.0	15.0
		15.0	13.0	14.0	14.0	18.0	17.5	19.0	21.0
9	One, May 16.........	.....	.....	.....	.....	.....	.....	.....	......
10	One, May 19.........	11.0	9.0	13.5	12.5	14.5	20.5	.....	......
		15.0	12.0	15.5	14.0	19.0	16.5	.....	......
		17.0	11.0	15.5	15.0	22.0	20.5	.....	......
		14.5	14.5	9.0	7.0	.....	.....	.....	......
11	One, May 19.........	.....	.....	22.0	19.5	25.0	30.0	29.0	31.5
		.....	.....	22.5	19.0	27.0	27.5	25.0	29.0
		.....	.....	18.5	18.5	23.0	22.5	23.0	28.0
		.....	.....	22.0	19.5	21.0	23.0	23.0	33.0
12	One, May 19.........	26.5	23.0	28.5	26.0	34.5	37.0	36.5	41.0
		25.0	23.0	29.5	28.5	32.5	38.0	35.0	42.0
	APTEROUS FEMALES OF SECOND GENERATION:								
13	One, June 13.........	16.0	15.0	.....	.....	.....	.....	.....	......
14	One, June 13.........	17.5	15.5	.....	.....	.....	.....	.....	......
15	One, June 13.........	23.5	21.5	29.0	28.0	36.5	36.5	39.0	42.0
		.....	.....	30.0	28.5	33.0	35.5	36.5	40.0
16	One, June 13.........	25.0	22.0	30.5	29.5	35.0	35.5	38.0	42.5
		25.5	21.5	30.0	29.0	35.5	37.0	37.0	41.5
17	One, June 13.........	20.0	17.5	.....	.....	.....	.....	.....	......
		17.5	15.0	.....	.....	.....	.....	.....	......
	COLONIES:								
18	One, June 21.........	19.0	16.0	31.5	29.5	36.5	38.0	38.0	42.5
		27.5	24.5	.....	.....	.....	.....	.....	......
19	One, June 21.........	26.0	22.5	30.0	28.0	34.0	36.0	38.0	41.5
		24.5	22.5	28.0	28.5	37.0	37.5	34.0	40.5
20	One, June 22.........	19.5	16.5	33.0	31.5	39.5	40.5	43.0	47.0
		27.0	25.5	.....	.....	.....	.....	.....	......
21	One, June 22.........	19.0	16.5	28.0	28.5	37.0	39.0	35.5	43.0
		19.0	15.0	30.5	30.0	32.0	37.0	34.5	41.0
		25.0	24.0	.....	.....	.....	.....	.....	......
		24.0	23.0	.....	.....	.....	.....	.....	......
22	One, June 29.........	.....	.....	30.5	30.5	36.5	39.5	36.5	41.0
		.....	.....	25.0	24.0	28.0	28.0	39.0	44.0
		.....	.....	29.0	28.0	34.0	35.0	30.0	32.0

TABLE I.— MEASUREMENTS OF FRUITS ATTACKED BY *A. sorbi* (*continued*).

No. of cluster.	NUMBER OF APHIDES.	AUG. 7.		AUG. 11.		AUG. 18.		AUG. 25.	
		Axial.	Trans.	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	STEM-MOTHERS:	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Five, May 8	.....	.....	.....	.....	.....	.....	.....	.....
		.....	.....	.....	.....	.....	.....	.....	.....
2	Twenty-five, May 13	.....	.....	.....	.....	.....	.....	.....	.....
3	Ten, May 15	.....	.....	.....	.....	.....	.....	.....	.....
4	Ten, May 15	.....	.....	.....	.....	.....	.....	.....	.....
5	Ten, May 15	.....	.....	.....	.....	.....	.....	.....	.....
6	Eight, May 15	.....	.....	.....	.....	.....	.....	.....	.....
7	One, May 16	38.0	42.0	38.0	45.5	40.0	47.0	39.5	47.5
		36.0	44.0	35.0	41.0	41.5	47.0	42.5	46.5
		34.0	39.5	41.0	45.5	37.0	45.0	38.5	45.0
8	One, May 16	20.5	25.0	24.5	25.0	21.5	22.0	.....	.....
		23.0	24.5	22.5	22.0	22.0	25.0	.....	.....
		20.0	21.0	21.5	22.0	25.0	26.5	.....	.....
		19.0	21.0	21.0	22.0	23.0	24.0	.....	.....
		22.0	23.0	.....	.....	.....	.....	.....	.....
9	One, May 16	.....	.....	.....	.....	.....	.....	.....	.....
10	One, May 19	.....	.....	.....	.....	.....	.....	18.0	20.5
		.....	.....	.....	.....	.....	.....	20.5	21.5
		.....	.....	.....	.....	.....	.....	28.0	30.5
		.....	.....	.....	.....	.....	.....	.....	.....
11	One, May 19	29.0	33.5	30.5	33.5	30.0	34.0	31.5	34.0
		26.0	32.0	27.0	34.5	28.0	36.0	29.0	37.0
		24.0	31.0	26.0	31.0	25.5	28.0	22.0	28.5
		24.0	33.0	.....	.....	.....	.....	.....	.....
12	One, May 19	38.0	45.0	39.0	46.0	38.0	46.5	40.0	45.5
		37.5	45.0	38.0	46.0	36.0	47.0	35.5	46.0
	APTEROUS FEMALES OF SECOND GENERATION:								
13	One, June 13	.....	.....	.....	.....	.....	.....	.....	.....
14	One, June 13	.....	.....	.....	.....	.....	.....	.....	.....
15	One, June 13	39.0	42.0	37.5	44.0	42.0	47.5	42.0	51.0
		37.5	44.0	39.0	47.0	38.5	45.5	39.0	45.0
16	One, June 13	38.5	43.0	40.5	47.0	43.0	49.0	42.5	51.5
		37.5	42.0	.....	.....	.....	.....	.....	.....
17	One, June 13	.....	.....	.....	.....	.....	.....	.....	.....
		.....	.....	.....	.....	.....	.....	.....	.....
	COLONIES:								
18	One, June 21	38.5	45.0	41.5	45.0	44.0	48.5	44.0	50.5
		.....	.....	.....	.....	.....	.....	.....	.....
19	One, June 21	.....	.....	.....	.....	.....	.....	.....	.....
		.....	.....	.....	.....	.....	.....	.....	.....
20	One, June 22	43.0	50.0	45.0	52.0	47.0	56.0	47.0	57.5
		.....	.....	.....	.....	.....	.....	.....	.....
21	One, June 22	37.0	44.0	37.5	46.0	39.0	46.0	.....	.....
		35.5	43.0	37.0	45.0	39.5	48.0	.....	.....
		.....	.....	.....	.....	.....	.....	.....	.....
		.....	.....	.....	.....	.....	.....	.....	.....
22	One, June 29	36.5	46.5	38.5	46.5	40.0	46.5	36.0	42.5
		39.0	44.0	32.0	38.0	35.0	41.0	.....	.....
		30.5	35.0	.....	.....	.....	.....	.....	.....

TABLE I.— MEASUREMENTS OF FRUITS ATTACKED BY *A. sorbi* (*concluded*).

No. of cluster.	NUMBER OF APHIDES.	SEPT. 1.		SEPT. 7.		SEPT. 16.		SEPT. 30.	
		Axial.	Trans.	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	STEM-MOTHERS:	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Five, May 8	.....	.....	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	.....	.....	......
2	Twenty-five, May 13	.....	.....	.....	.....	.....	.....	.....	......
3	Ten, May 15	.....	.....	.....	.....	.....	.....	.....	......
4	Ten, May 15	.....	.....	.....	.....	.....	.....	.....	......
5	Ten, May 15	.....	.....	.....	.....	.....	.....	.....	......
6	Eight, May 15	.....	.....	.....	.....	.....	.....	.....	......
7	One, May 16	41.5	49.0	.....	.....	42.0	50.0	42.0	50.0
		44.0	50.5	.....	.....	45.0	54.5	45.5	56.0
		41.0	49.0	.....	.....	41.0	51.5	41.5	51.0
8	One, May 16	.....	.....	.....	.....	20.5	27.5	20.0	28.0
		.....	.....	.....	.....	21.0	30.5	21.5	31.0
		.....	.....	.....	.....	21.0	29.5	21.0	30.0
		.....	.....	.....	.....	20.5	25.5	20.5	26.0
		.....	.....	.....	.....	.....	.....	.....	......
9	One, May 16	.....	.....	.....	.....	.....	.....	.....	......
10	One, May 19	.....	.....	.....	.....	18.0	25.0	18.0	24.0
		.....	.....	.....	.....	20.5	23.0	20.5	22.5
		.....	.....	.....	.....	28.0	35.5	28.0	36.0
		.....	.....	.....	.....	.....	.....	.....	......
11	One, May 19	30.5	39.5	.....	.....	.....	.....	.....	......
		30.0	33.5	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	.....	.....	......
12	One, May 19	39.0	47.0	40.0	47.0	43.0	49.5	43.0	50.0
		36.0	45.0	36.5	45.5	35.5	47.0	35.5	46.0
	APTEROUS FEMALES OF SECOND GENERATION:								
13	One, June 13	.....	.....	.....	.....	.....	.....	.....	......
14	One, June 13	.....	.....	.....	.....	.....	.....	.....	......
15	One, June 13	43.0	53.5	45.0	55.0	49.5	55.5	49.5	59.0
		40.0	46.0	41.0	47.0	42.0	47.0	42.0	47.0
16	One, June 13	43.0	52.5	44.5	53.5	52.0	55.5	52.0	57.0
		.....	.....	.....	.....	.....	.....	.....	......
17	One, June 13	.....	.....	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	.....	.....	......
	COLONIES:								
18	One, June 21	45.0	50.5	45.0	55.0	50.0	57.5	50.0	59.5
		.....	.....	.....	.....	.....	.....	.....	......
19	One, June 21	.....	.....	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	.....	.....	......
20	One, June 22	49.0	58.5	50.0	60.0	54.0	61.0	54.0	62.5
		.....	.....	.....	.....	.....	.....	.....	......
21	One, June 22	39.5	49.0	39.5	49.5	44.5	54.0	44.5	57.0
		40.0	52.5	41.0	52.0	45.0	50.0	45.0	49.0
		.....	.....	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	.....	.....	......
22	One, June 29	36.5	43.5	37.5	46.5	40.0	48.5	40.0	50.0
		.....	.....	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	.....	.....	......

TABLE II.— MEASUREMENTS OF FRUITS ATTACKED BY *A. avenæ*.

No. of cluster.	NUMBER OF APHIDES.	JUNE 27.		JULY 5.		JULY 19.		JULY 29.	
		Axial.	Trans.	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	STEM-MOTHERS:	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Fifteen, May 9.......	25.0	23.0	29.0	29.0	33.5	36.5	36.0	42.0
		25.0	23.0	29.0	29.5	34.5	35.0	35.0	42.0
2	Sixty, May 10.......	.....	.....	26.0	25.5	31.0	34.5	38.0	44.0
		.....	.....	29.0	28.0	35.0	37.5	34.0	41.5
3	Forty-two, May 12...	18.0	15.0	.....	.....	.....	.....	.....	......
4	Thirty, May 12......	21.0	19.0	27.0	25.5	35.0	39.0	.....	......
		24.0	22.5	29.0	30.0	32.5	38.0	.....	......
5	Thirty, May 13......	22.0	23.0	26.0	29.0	32.5	35.0	32.0	41.5
		21.0	20.5	24.5	26.5	34.0	36.0	34.0	38.5
		21.5	20.0	24.5	25.0	32.5	36.5	34.5	38.5
6	Thirty, May 13......	18.0	15.5	29.0	31.0	33.0	39.5	33.5	42.0
		24.0	25.0	27.0	27.5	35.0	39.0	37.0	43.5
		23.5	22.0	29.0	30.5	31.5	33.5	32.0	37.0
		24.0	25.0	.....	.....	.....	.....	.....	......
7	Thirty, May 13......	22.5	19.5	28.0	27.0	33.0	35.0	37.5	42.0
		26.0	23.5	30.5	30.0	37.5	38.0	31.5	40.0
8	Thirty, May 13......	24.0	21.5	29.0	27.0	34.0	37.5	36.0	43.0
		25.0	23.0	28.0	29.0	35.5	36.0	37.0	42.0
9	Thirty, May 13......	19.0	14.0	.....	.....	.....	.....	.....	......

TABLE II.— MEASUREMENTS OF FRUITS ATTACKED BY *A. avenæ* (*continued*).

No. of cluster.	NUMBER OF APHIDES.	AUG. 7.		AUG. 11.		AUG. 18.		SEPT. 1.	
		Axial.	Trans.	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	STEM-MOTHERS:	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Fifteen, May 9.......	37.5	45.0	38.5	47.5	41.0	52.0	43.5	55.5
		36.5	45.0	37.0	47.0	42.0	52.0	43.5	56.0
2	Sixty, May 10........	.....	.....	.....	.....	40.0	53.0	43.5	54.5
		.....	.....	.....	.....	36.0	51.5	38.0	55.0
3	Forty-two, May 12...	.....	.....	.....	.....	.....	.....	.....	......
4	Thirty, May 12......	38.0	47.0	38.0	49.5	37.0	52.0	40.0	55.0
		34.5	47.5	36.5	46.5	38.5	52.0	41.5	57.0
5	Thirty, May 13......	35.5	43.0	.....	.....	.....	.....	.....	......
		34.5	39.0	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	.....	.....	......
6	Thirty, May 13......	34.0	45.0	36.0	48.5	39.5	52.0	40.0	57.0
		37.5	44.0	38.5	49.5	47.5	54.0	41.5	55.5
		33.0	41.0	35.0	43.0	36.5	46.5	38.0	48.5
		.....	.....	.....	.....	.....	.....	.....	......
7	Thirty, May 13......	39.0	45.0	38.5	48.0	35.0	50.0	36.0	54.0
		34.5	43.0	35.0	44.0	.....	.....	.....	......
8	Thirty, May 13......	37.0	45.0	39.0	47.0	39.5	50.0	.....	......
		.....	.....	.....	.....	.....	.....	.....	......
9	Thirty, May 13......	.....	.....	.....	.....	.....	.....	.....	......

TABLE II.— MEASUREMENTS OF FRUITS ATTACKED BY *A. avenæ* (*concluded*).

No. of cluster.	NUMBER OF APHIDES.	SEPT. 7.		SEPT. 16.		SEPT. 30.	
		Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	STEM-MOTHERS:	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Fifteen, May 9	44.0	56.0	48.5	60.0	51.0	63.0
		42.0	57.0	48.5	60.5	51.0	63.0
2	Sixty, May 10	44.0	56.0	46.5	59.0	.....	......
		39.0	54.0	48.5	59.0	.....	......
3	Forty-two, May 12	.....	.....	.....	.....	.....	......
4	Thirty, May 12	41.0	55.5	47.0	58.0	48.0	61.5
		42.0	58.0	49.0	60.5	50.0	64.0
5	Thirty, May 13	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	......
6	Thirty, May 13	40.5	57.5	49.5	61.5	50.0	62.0
		42.0	56.0	50.0	60.5	45.0	55.5
		39.0	50.0	47.5	52.5	.....	......
		.....	.....	.....	.....	40.0	61.0
7	Thirty, May 13	37.0	55.0	45.5	60.0	.....	......
		.....	.....	.....	.....	.....	......
8	Thirty, May 13	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	......
9	Thirty, May 13	.....	.....	.....	.....	.....	......

TABLE III.— MEASUREMENTS OF FRUITS ATTACKED BY *A. pomi*.

No. of cluster.	NUMBER OF APHIDES.	JUNE 27.		JULY 5.		JULY 19.		JULY 29.	
		Axial.	Trans.	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	STEM-MOTHERS:	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Forty to sixty, May 19.	13.0	11.5	.....	.....	.....	.....	.....	......
		13.0	13.5	.....	.....	.....	.....	.....	......
2	Same	18.0	14.0	19.0	15.0	.....	.....	.....	......
3	Same	.....	.....	.....	.....	.....	.....	.....	......
4	Same	17.0	14.0	.....	.....	.....	.....	.....	......
		16.5	16.0	.....	.....	.....	.....	.....	......
5	Same	14.0	14.0	.....	.....	.....	.....	.....	......
6	Same	17.0	15.0	.....	.....	.....	.....	.....	......
		19.5	15.0	.....	.....	.....	.....	.....	......
	COLONY:								
7	One, June 29	.....	.....	28.5	28.0	35.0	38.0	36.0	44.5
		.....	.....	29.5	30.5	35.0	35.0	36.0	40.0

TABLE III.— MEASUREMENTS OF FRUITS ATTACKED BY *A. pomi* (*continued*).

No. of cluster.	NUMBER OF APHIDES.	AUG. 11.		AUG. 18.		SEPT. 1.	
		Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	STEM-MOTHERS:	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Forty to sixty, May 19	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	......
2	Same	.....	.....	.....	.....	.....	......
3	Same	.....	.....	.....	.....	.....	......
4	Same	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	......
5	Same	.....	.....	.....	.....	.....	......
6	Same	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	......
	COLONY:						
7	One, June 29	38.0	45.5	41.5	52.0	42.5	58.5
		39.0	52.0	39.5	48.0	42.0	54.0

TABLE III.— MEASUREMENTS OF FRUITS ATTACKED BY *A. pomi* (*concluded*).

No. of cluster.	NUMBER OF APHIDES.	SEPT. 7.		SEPT. 16.		SEPT. 30.	
		Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	STEM-MOTHERS:	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	Forty to sixty, May 19	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	......
2	Same	.....	.....	.....	.....	.....	......
3	Same	.....	.....	.....	.....	.....	......
4	Same	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	......
5	Same	.....	.....	.....	.....	.....	......
6	Same	.....	.....	.....	.....	.....	......
		.....	.....	.....	.....	.....	......
	COLONY:						
7	One, June 29	43.0	59.0	49.0	60.0	49.0	60.0
		42.5	55.0	47.5	58.0	.....	......

TABLE IV.— MEASUREMENTS OF APPLE DIAMETERS ON CHECKS.

No. of Cluster.	June 27.		July 5.		July 19.		July 29.	
	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	18.0	15.0	30.5	30.5	38.0	40.0	40.0	45.0
	25.0	24.0	.....	.....	.....	.....	.....	......
2	25.5	24.0	27.0	29.5	32.0	37.0	39.0	43.0
	21.5	23.0	27.0	28.5	37.5	38.0	35.0	42.5
3	23.5	22.5	29.0	28.0	34.0	35.0	.....	......
	23.5	20.5	28.0	26.0	36.0	36.5	.....	......
4	25.0	25.0	29.0	30.0	.....	.....	36.5	42.0
	27.0	25.0	30.0	30.0	.....	.....	37.0	43.0
5	25.0	25.0	30.0	31.5	35.0	40.0	40.0	47.5
	27.5	27.0	32.0	33.0	35.0	38.5	37.0	44.0
6	22.5	23.5	32.5	34.0	.....	.....	40.0	50.0
	27.5	27.5	29.0	30.5	.....	.....	35.0	45.0
7	20.0	18.0	31.5	32.5	32.0	38.0	35.0	43.0
	24.0	23.0	26.5	28.5	36.0	41.5	37.0	48.0
	27.5	27.5	.....	.....	.....	.....	.....	......
8	23.5	22.0	26.5	28.0	34.0	35.5	35.0	40.5
	25.0	23.0	27.5	28.5	32.0	35.0	37.0	41.5
9	23.0	22.0	28.5	29.0	33.5	38.0	36.0	42.5
	25.0	22.5	28.5	28.5	34.0	36.5	34.0	42.0
10	24.0	23.0	28.0	30.5	33.5	39.0	36.0	46.0
	21.5	20.0	.....	.....	.....	.....	.....	......

TABLE IV.— MEASUREMENTS OF APPLE DIAMETERS ON CHECKS (*continued*).

NO. OF CLUSTER.	AUG. 7.		AUG. 11.		AUG. 18.		SEPT. 1.	
	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1............	42.5	49.0	44.5	51.0	45.5	54.0	48.5	58.0
	.....	.....	.....	.....	.....	.....	.....	......
2............	42.0	47.0	43.0	47.5	43.0	48.5	39.5	51.0
	39.0	45.0	39.5	47.5	47.5	48.0	45.0	52.0
3............	38.0	46.0	.....	.....	.....	.....	43.5	54.0
	38.0	45.5	.....	.....	.....	.....	45.0	55.0
4............	.....	.....	.....	.....	37.5	50.0	40.5	53.0
	.....	.....	.....	.....	42.0	53.0	44.0	55.5
5............	.....	.....	40.0	50.0	43.5	57.0	46.5	60.0
	.....	.....	42.0	51.5	42.0	52.0	.....	......
6............	42.0	52.0	42.5	52.5	41.5	54.0	41.5	57.5
	37.0	46.5	38.0	51.0	40.5	55.0	.....	......
7............	38.0	51.0	40.0	54.0	39.5	50.0	40.5	54.0
	37.0	45.0	37.5	47.5	41.5	57.0	45.0	60.0
	.....	.....	.....	.....	.....	.....	.....	......
8............	.....	.....	.....	.....	.....	.....	.....	......
	.....	.....	.....	.....	.....	.....	.....	......
9............	.....	.....	.....	.....	.....	.....	.....	......
	.....	.....	.....	.....	.....	.....	.....	......
10............	.....	.....	38.0	40.0	40.0	54.0	43.0	56.5
	.....	.....	.....	.....	.....	.....	.....	.....

TABLE IV.—MEASUREMENTS OF APPLE DIAMETERS ON CHECKS (*concluded*).

No. of Cluster.	Sept. 7.		Sept. 16.		Sept. 30.	
	Axial.	Trans.	Axial.	Trans.	Axial.	Trans.
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	49.0	60.0	51.0	61.0	51.5	64.0
	......	......	......	......	......	....
2	40.0	51.5	46.0	52.5	46.0	54.0
	46.0	52.5	......	......	......	....
3	44.0	55.0	......	......	49.5	61.5
	46.5	57.0	......	......	......	....
4	41.0	55.0	......	......	48.0	58.0
	45.0	56.0	......	......	......	....
5	47.0	61.5	52.0	65.0	54.0	69.0
	......	......	......	......	......	....
6	42.5	59.0	50.5	64.5	52.5	66.0
	......	......	......	......	......	....
7	42.0	57.0	46.5	62.0	47.5	64.0
	45.5	61.0	52.5	67.0	55.0	70.0
	......	......	......	......	......	....
8	......	......	......	......	......	....
	......	......	......	......	......	....
9	......	......	......	......	......	....
	......	......	......	......	......	....
10	44.5	58.5	......	......	......	....
	......	......	......	......	......	....

TABLE V.—SUMMARY OF TABLE I, SHOWING EFFECTS OF *A. sorbi* ON GROWTH OF APPLES.

DATE OF MEASUREMENT.	Number of fruit clusters.	NUMBER OF APPLES.		AXIAL DIAMETER.		TRANSVERSE DIAMETER.	
		Total.	Average per cluster.	Total.	Average.	Total.	Average.
				Mm.	*Mm.*	*Mm.*	*Mm.*
June 27.........	16	35	2.19	668.5	19.1	590.0	16.8
July 5.........	13	32	2.46	752.0	23.5	719.5	22.5
19.........	12	30	2.5	870.0	29.0	904.5	30.1
29.........	11	27	2.4	839.5	31.1	952.0	35.3
Aug. 7.........	10	25	2.5	803.5	32.1	938.0	37.5
11.........	10	21	2.1	712.5	33.9	824.5	39.2
18.........	10	21	2.1	735.5	35.0	856.0	40.7
25.........	9	17	1.9	595.5	35.0	700.5	41.2
Sept. 1.........	9	15	1.7	598.0	39.8	719.5	47.9
7.........	7	10	1.4	420.0	42.0	511.0	51.1
16.........	10	20	2.0	733.0	36.6	878.0	43.9
30.........	10	20	2.0	734.0	36.7	891.5	44.5

TABLE VI.—SUMMARY OF TABLE II, SHOWING EFFECTS OF *A. avenæ* ON GROWTH OF APPLES.

DATE OF MEASUREMENT.	Number of fruit clusters.	NUMBER OF APPLES.		AXIAL DIAMETER.		TRANSVERSE DIAMETER.	
		Total.	Average per cluster.	Total.	Average.	Total.	Average.
				Mm.	*Mm.*	*Mm.*	*Mm.*
June 27.........	8	17	2.1	383.5	22.5	355.0	20.9
July 5.........	7	16	2.3	444.5	27.7	450.0	28.1
19.........	7	16	2.3	540.0	33.7	586.5	36.6
29.........	6	14	2.3	488.0	34.8	577.5	41.2
Aug. 7.........	6	12	2.0	431.5	35.9	529.5	44.1
11.........	5	10	2.0	372.0	37.2	470.5	47.0
18.........	6	11	1.8	432.5	39.3	565.0	51.4
Sept. 1.........	5	10	2.0	405.5	40.5	548.0	54.8
7.........	5	10	2.0	410.5	41.0	555.0	55.5
16.........	5	10	2.0	480.5	48.0	591.5	59.1
30.........	4	7	1.7	335.0	47.9	430.0	61.4

Table VII.— Summary of Table III, Showing Effects of *A. pomi* on Growth of Apples.

Date of Measurement.	Number of fruit clusters.	Number of Apples.		Axial Diameter.		Transverse Diameter.	
		Total.	Average per cluster.	Total.	Average.	Total.	Average.
				Mm.	Mm.	Mm.	Mm.
June 27	5	8	1.6	128.0	16.0	113.0	14.1
July 5	2	3	1.5	77.0	25.4	73.5	24.5
19	1	2	2.0	70.0	35.0	73.0	36.5
29	1	2	2.0	72.0	36.0	84.5	42.2
Aug. 11	1	2	2.0	77.0	38.5	97.5	48.7
18	1	2	2.0	81.0	40.5	100.0	50.0
Sept. 1	1	2	2.0	84.5	42.2	112.5	56.2
7	1	2	2.0	85.5	42.7	114.0	57.0
16	1	2	2.0	96.5	48.2	118.0	59.0
30	1	1	1.0	49.0	49.0	60.0	60.0

Table VIII.— Summary of Table IV, Showing Measurements of Apple Diameters on the Checks.

Date of Measurement.	Number of fruit clusters.	Number of Apples.		Axial Diameter.		Transverse Diameter.	
		Total.	Average per cluster.	Total.	Average.	Total.	Average.
				Mm.	Mm.	Mm.	Mm.
June 27	10	21	2.1	505.0	24.0	483.0	23.0
July 5	10	18	1.8	521.0	28.9	537.0	29.8
19	8	14	1.7	482.5	34.4	528.5	37.7
29	9	16	1.8	589.5	36.8	705.5	44.1
Aug. 7	5	9	1.8	353.5	39.2	427.0	47.4
11	6	10	1.7	405.0	40.5	492.5	49.2
18	7	12	1.7	504.0	42.0	632.5	52.7
Sept. 1	8	12	1.5	522.5	43.5	666.5	55.5
7	8	12	1.5	533.0	44.4	684.0	57.0
16	5	6	1.2	298.5	49.7	372.0	62.0
30	7	8	1.1	404.0	50.5	506.5	63.3

As a result of the rapid multiplication of the aphides, the infested clusters were soon completely overrun by them. The species *avenæ* reached the height of its abundance earlier than either *sorbi* or *pomi*, and was likewise the first to show retrogression in numbers after reaching the point of maximum reproduction. Early in June the insects abandoned the fruit clusters and, in their efforts to migrate, collected on the netting, where they died. By late June the force of the attack by *sorbi* had spent itself, while *pomi* remained on the clusters thruout the summer, altho the numbers were decreased during midsummer.

COMPARISON OF INJURIES BY THE DIFFERENT APHIDES.

The most severe injury was shown with the early infested clusters attacked by *sorbi* and those infested with *pomi*. Clusters one to six, inclusive, of the *sorbi* series and the same lots of the *pomi* series were so heavily coated with the aphides that they soon became a sticky, sooty mass; and the fruits were completely destroyed.

The attack by *avenæ*, while of shorter duration, was apparently no less intense while it lasted; for the insects clustered on practically the entire surfaces of the infested clusters, the leaves, stems, developing fruits, and even the petals being overrun with them. Nevertheless, the injurious effects of the feeding by this species were much less obvious than with the other species. Injury by *avenæ* was evidenced by a gentle curling of the leaves, and in some cases by a slightly one-sided growth of the fruits. At an early date many of the affected fruits showed a distinct stippling with minute black points and there was also a brownish discoloration on the upper surfaces, which may have been due to some other cause and is as yet not clearly understood.

By the last of May the clusters infested with *sorbi* showed considerable curling of the foliage; and at this time, even before all of the petals had dropped, there was in some cases a marked curling of the fruit stems and contortion of the young fruits. The curling of the foliage became serious early in June, and by this time many of the fruits and fruit stems had become badly distorted. At this date *pomi* had not caused such noticeable injury to the fruits. As the infestation continued the damage grew more striking, and on June 22 most of the fruits of the *sorbi* and *pomi* series were badly injured. A number of the clusters of the *sorbi* lot were so seriously affected that the fruit was entirely destroyed. The contortion of the fruits, due to *sorbi*, frequently took the form of a reduction in growth on one side, resulting in a curving of the axial diameter, frequently accompanied by more or less protrusion of the calyx, with a star-shaped series of prominent ridges radiating from the center of the calyx end. Contortion caused by *pomi* was quite similar, but seemed to be characterized by a greater tendency for the entire fruit to become angulate, with longitudinal elevations and depressions.

At the time of picking of the fruit, the apples of the *avenæ* series showed no contortion, altho in one case the fruit was slightly misshaped. Most of these fruits were poorly colored, showing a yellowish-green hue. Several displayed a brownish discoloration on one side, which may have been due to some other factor, while nearly all of the apples were more or less stippled with minute black points.

The majority of the fruits of the *pomi* and *sorbi* series which had undergone extreme contortion dropped before the fruit was picked. The one fruit of the *pomi* lot was about normal in shape, but was rather pale in color, and was slightly stippled with the fine black points, as has been previously noted. The apples of the *sorbi* series, at the time of picking, while not showing the most extreme type of distortion which is sometimes found, were more generally ill-shaped than those of the other series. The injury by this species was usually indicated by the protuberance of the calyx end with a star-shaped series of elevations radiating from the calyx cup. In less severe cases the calyx basin and the depression around the stem seemed abnormally shallow and flat bottomed. As was the case in the other series subject to attacks by aphides, these fruits were poorly colored and frequently stippled with fine black dots.

EFFECTS OF ATTACKS ON SETTING OF APPLES.

As was brought out in the discussion of last year's studies, the attack of *sorbi* was found to increase the average number of fruits per cluster, resulting in the clusters of so-called "aphis apples" which are frequently seen in infested orchards. An examination of Table I will show that the severe and early infestation of the first six clusters resulted in the dropping of the fruits. This was apparently a destruction of the young fruit, however, rather than a failure of the fruit to set. In the case of clusters Nos. 7, 8, 10, 11 the infestation seems to have become severe before the normal number of fruits of the cluster had dropped, and yet the attacks were not so injurious as completely to destroy the fruits. As a consequence the normal reduction of the numbers of the fruits commonly known as the "June drop" did not occur, with the result that there were from three to five rather small apples to each cluster. Later infestation did not prevent the usual reduction in numbers of the fruits, and smaller clusters therefore resulted, which in some measure resisted the effects of the aphides as far as premature dropping was concerned. Of the clusters infested with *pomi* there was only one in which the fruits were not completely destroyed, and in this instance the cluster contained only two apples. It seems quite probable, however, that had the early infestation been less rigorous, larger numbers of apples per cluster would have remained in this lot. The average number of apples per cluster was also somewhat raised in the *avenæ* series. However, it appears that there was less tendency to form large clusters in this series than was the case with the *sorbi* series.

EFFECTS OF ATTACKS ON GROWTH OF APPLES.

The attacks of all species of the aphides included in this study resulted in a diminution of the size of the fruits that were infested. The greatest inhibition in growth occurred in the series subject to attack by *sorbi*, which is graphically compared with the growth of the check fruits in Fig. 8. It will be noted that early in the season the axial diameters were greater than the transverse measurements. The transverse diameters increased more rapidly, however, and soon became greater than the axial diameters. In the check series the two diameters were approximately equal on June 30, while the *sorbi* series were equal about July 12. In other words, the aphis attack inhibited the growth of the transverse diameter to a greater extent proportionally than that of the axial diameter. This was found to be true in the case of each species and varies directly with the amount of injury caused by the species making the attack.

Similar curves of growth for the apples of the series affected by *avenæ* and *pomi* would occupy positions intermediate between the curves here shown.

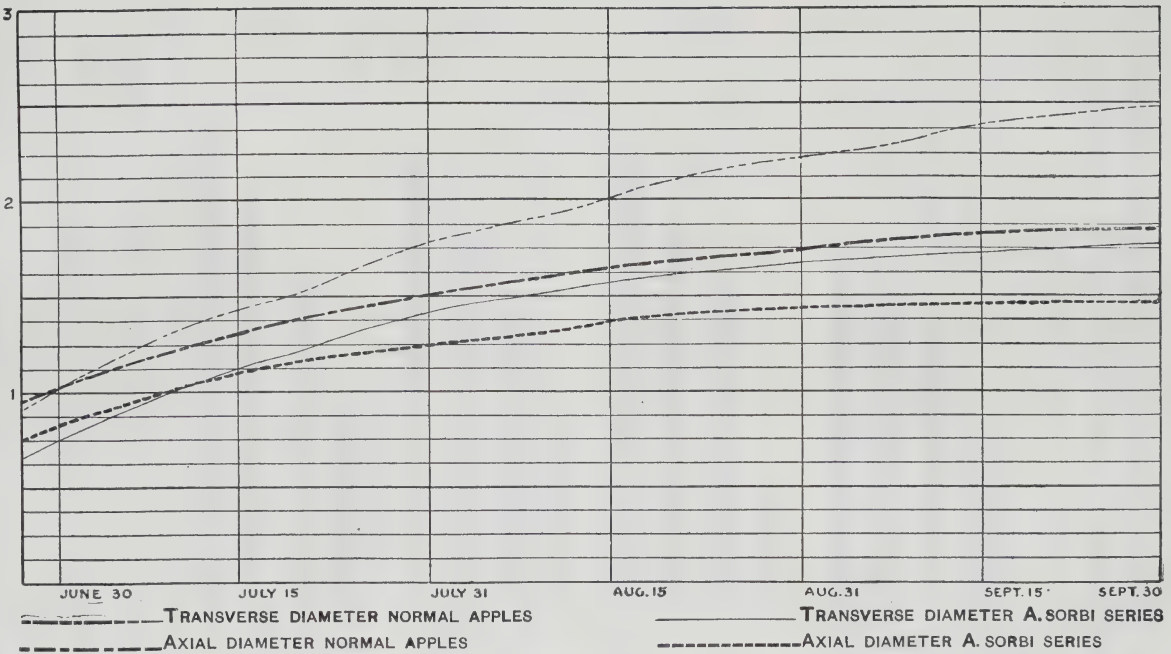


Fig. 8.—Diagram Showing Growth of Apples Infested with Rosy Aphis in Comparison with Normal Fruit.

Figure 9 represents approximately the average monthly sizes of the apples of the several series. It will be seen that the fruits of the *sorbi* lot suffered the greatest reduction in dimensions. Those of the *avenæ* series closely approach the checks in size, while the *pomi* series are slightly smaller. These measurements should scarcely be regarded as typical of the attacks of *pomi*, for an examination of

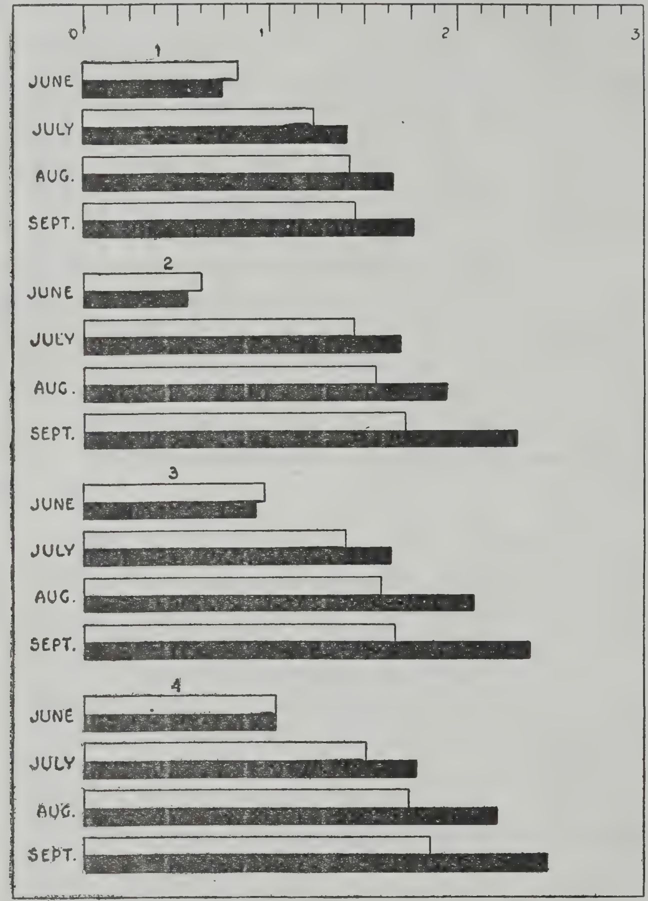


FIG. 9.—DIAGRAM SHOWING AVERAGE GROWTH OF INFESTED AND SOUND APPLES.

1, *Sorbi* series; 2, *pomi* series; 3, *avenæ* series; and 4, checks. White bars, axial diameter; black bars, transverse diameter.

Table III will show that only one of the affected clusters retained fruits throughout the season. This cluster was infested late in the season, and did not suffer such severe injury as would have been caused by an earlier infestation.

TEST WITH LIME-SULPHUR AND NICOTINE SOLUTION.

This test was conducted in an orchard of mixed varieties of apples, containing about one hundred and seventy-six trees, of which one-third or more were of the variety Rome. The latter are twenty-one years old and, because of their size, as well as susceptibility to the rosy aphis, lend themselves well to the proposed experiment.

PLAN OF EXPERIMENT.

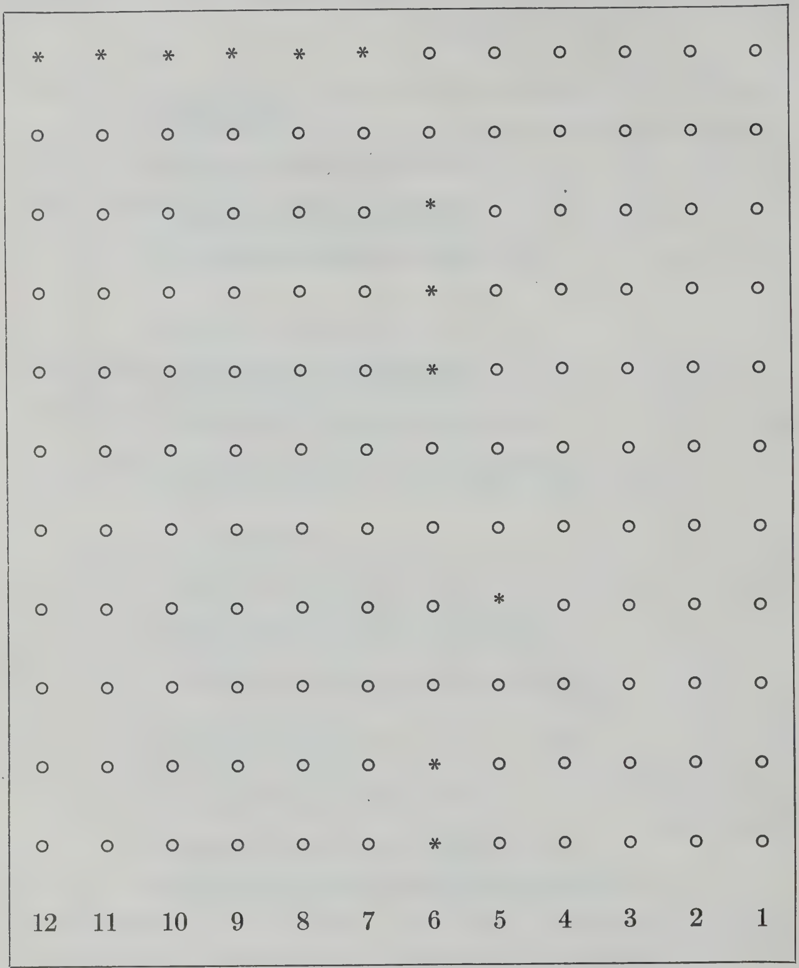
In planning the test the chief object was to determine the effects of lime-sulphur and nicotine solution against the newly-hatched nymphs of the rosy aphis. Plats were provided as follows:

Plat I. Nicotine solution (40 per ct.) three-fourths of a pint to one hundred gallons of lime-sulphur solution, 1 to 8, applied as a delayed dormant treatment.
Plat II. Check.

The trees in Plat I, rows 1 to 5 inclusive, Fig. 10, were treated with nicotine solution combined with lime-sulphur on May 1, when leaves of the most advanced buds were projecting as much as one-fourth to half an inch. The spraying was purposely delayed until the eggs had begun to hatch and it was reasonably certain that most of the nymphs had emerged. As in all of our experiments against this pest the mixture was applied in liberal quantities and no tree was considered properly sprayed until every bud was wetted with the liquid. In this operation an average of eleven gallons of material was applied to each tree.

RESULTS OF SPRAYING ON APHIDES AND TREES.

Unlike last season's experience, *avenæ* was very scarce in the experimental plats and the occasional specimens that were observed were discovered only by careful searching. During the earlier part of the season *sorbi* was the dominant species, and it was not especially difficult to find at least one, and occasionally three or more of the nymphs on some of the buds. The injury to the crop was largely if not entirely the result of attacks by this species. The associated form, *pomi*, became abundant during June and many of the tips of the new growth were abundantly infested with the species, while fruit clusters were exempt from injury. On June 30, when *sorbi* had ceased breeding on the apples and the migrants had largely left the orchard, each plat was carefully examined to note the number of affected fruits. The data are tabulated in Table IX.



* Check, Plat 2. ° Sprayed, Plat 1, rows 1 to 5.

FIG. 10.— DIAGRAM OF ORCHARD SHOWING SPRAYED AND CHECK TREES.

TABLE IX.— RESULTS OF SPRAYING AGAINST ROSY APHIS.

VARIETY.	LOCATION.	Treatment.	NUMBER OF DIFFERENT SECTIONS AFFECTED.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row. Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.			
Rome.......	1— 1	Lime-sulphur and nicotine.	1	0	0	0	1	2	0
Husband....	1— 2		0	0	0	0	0	0	0
Rome.......	1— 3		0	0	0	0	0	0	0
Axident.....	1— 4		0	0	0	0	0	0	0
Rome.......	1— 5		0	0	0	0	0	0	0
Hanlon......	1— 6		0	0	0	0	0	0	0
Rome.......	1— 7		0	0	0	0	0	0	0
x...........	1— 8		0	0	0	0	0	0	0
Rome.......	1— 9		0	0	0	0	0	0	0
x...........	1—10		0	0	0	0	0	0	0
Rome.......	1—11		0	0	0	0	0	0	0
Rome.......	2— 1		0	0	0	0	0	0	0
x...........	2— 2		0	0	0	0	0	0	0
Rome.......	2— 3		0	0	0	0	0	0	0
Florence.....	2— 4		0	0	0	0	0	0	0
Rome.......	2— 5		0	0	0	0	0	0	0
x...........	2— 6		0	0	0	0	0	0	0
Rome.......	2— 7		0	0	0	0	0	0	0
Wagener.....	2— 8		0	0	0	0	0	0	0
Rome.......	2— 9		0	0	0	0	0	0	0
Kinnaird.....	2—10		0	0	0	0	0	0	0
Rome.......	2—11		0	0	0	0	0	0	0
Rome.......	3— 1		0	0	0	0	0	0	0
Kinnaird.....	3— 2		0	0	0	0	0	0	0
Rome.......	3— 3		0	0	0	0	0	0	0
Hubbardston.	3— 4		0	0	0	0	0	0	0
Rome.......	3— 5		0	0	0	0	0	0	0
Hubbardston.	3— 6		0	0	0	0	0	0	0
Rome.......	3— 7		0	0	0	0	0	0	0
Wagener.....	3— 8		0	0	0	0	0	0	0
Rome.......	3— 9		0	0	0	0	0	0	0
o...........	3—10								
Rome.......	3—11		0	0	0	0	0	0	0
Rome.......	4— 1		0	0	0	0	0	0	0
Husband....	4— 2		0	0	0	0	0	0	0
Rome.......	4— 3		0	0	0	0	0	0	0
Wheeler.....	4— 4		0	0	0	0	0	0	0
Rome.......	4— 5		0	0	0	0	0	0	0
A. Caville....	4— 6		0	0	0	0	0	0	0
Rome.......	4— 7		0	0	0	0	0	0	0
x...........	4— 8		0	0	0	0	0	0	0
Rome.......	4— 9		0	0	0	0	0	0	0

---

o Dead tree.
x Small tree.

TABLE IX.— RESULTS OF SPRAYING AGAINST ROSY APHIS (*concluded*).

VARIETY.	LOCATION.		Treatment.	NUMBER OF DIFFERENT SECTIONS AFFECTED.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row.	Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.			
Constantine..	4	10	Lime-sulphur and nicotine.	0	0	0	0	0	0	0
Rome.......	4	11		0	0	0	0	0	0	0
Rome.......	5	1		0	0	0	0	0	0	0
A. Caville....	5	2		0	0	0	0	0	0	0
Rome.......	5	3		0	0	0	0	0	0	0
*Westchester.	5	4		2	4	1	0	7	8	29
Rome.......	5	5		0	0	0	0	0	0	0
Westchester..	5	6		0	0	0	0	0	0	0
Rome.......	5	7		0	0	0	0	0	0	0
x..........	5	8		0	0	0	0	0	0	0
Rome.......	5	9		0	0	0	0	0	0	0
x..........	5	10		0	0	0	0	0	0	0
Rome.......	5	11		0	0	0	0	0	0	0
Rome.......	6	1	Checks	2	53	0	3	58	4	231
Rome.......	6	2		10	11	6	12	39	25	169
Lady Sweet..	6	7		0	0	2	4	6	0	24
Lady Sweet..	6	8		0	1	0	3	4	0	16
Rome.......	6	9		15	68	6	13	102	51	416
Rome.......	7	11		30	78	15	7	130	50	486
Rome.......	8	11		15	32	4	0	51	47	106
Rome.......	9	11		46	8	0	0	54	77	144
Rome.......	10	11		77	4	0	0	81	106	248
Rome.......	11	11		31	4	0	0	35	53	118
Rome.......	12	11		39	5	0	0	44	105	148

* Purposely left unsprayed for comparison with Tree 6, row 5.
x Small tree.

DISCUSSION OF RESULTS.

Previous to the initiation of our studies knowledge on the early activities of the rosy aphis was very meagre. Particularly was this true regarding the period when the species hatches and seeks the opening buds. This lack of information was largely due to the fact that the newly-hatched nymph was not known and was therefore unrecognizable from those of the associated species of aphides. Since the nymph of the first instar of *sorbi* has been discovered and is now distinctly differentiated from other forms, it has been possible to follow its activities quite closely; and both observations and experimental tests showed that as far as this orchard is concerned hatching was concluded on or a little before April 30. At this time the leaves of the most advanced blossom buds were projecting as much as half an inch.

The susceptibility of the rosy aphis to the delayed dormant application of lime-sulphur and nicotine solution is plainly indicated. Rarely in experiments with common orchard insects do sharper contrasts appear than existed between Plats I and II, with respect to this species. Altho the infestation was not large, the trees in Plat I were practically exempt from injuries either to the fruit or foliage, a condition quite different from that which existed in Plat II. While it is desirable to repeat these tests over a period of several years before drawing final conclusions, these results certainly border on the line of conclusive demonstration and are very convincing, especially when considered in the light of the data obtained in similar experiments during the preceding year.

AUXILIARY EXPERIMENTS DURING 1916.

Thru the courtesy of Commissioner Charles S. Wilson the auxiliary experiments were conducted with the assistance of Mr. L. F. Strickland, Inspector of the State Bureau of Horticulture for Niagara County, and Mr. A. B. Buchholz, Horticultural Inspector for Orleans County.

EXPERIMENTS IN NIAGARA COUNTY.

Test 1. Orchard of E. S. Gifford, Gasport.— In this planting thirty trees of the variety Greening, about sixty-one years old, were reserved for the test. On April 26, as the buds were green at the tips and occasional young leaves were expanded, the trees were sprayed with lime-sulphur in combination with nicotine sulphate (Black Leaf 40) in the usual proportions. In order to insure thoro treatment the trees were drenched with the spraying mixture, using on an average twenty-five gallons of the liquid to the tree.

Rosy and oat aphides were the more abundant species on the trees, the infestation of which was variable, averaging from one to seventeen of the insects to each bud. On May 12 and 13 the aphides were decidedly more abundant on the unsprayed Greenings. An examination of the unsprayed trees on June 21 showed that a considerable number of the fruit and leaf clusters had been injured by the rosy aphis. In the sprayed plat on this date eighteen trees were entirely free from damage by the rosy aphides, and twelve trees had a small number of injured clusters that were largely confined to areas which apparently were not reached by the spray. The conditions of the sprayed and check trees as regards injuries are given in Table X.

TABLE X.— RESULTS OF SPRAYING AGAINST ROSY APHIS.

Gifford orchard.

VARIETY.	LOCATION. Row, Tree.	Treatment.	NUMBER OF DIFFERENT SECTIONS AFFECTED. Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.	Centers of infestation.	Fruits injured.	Leaves curled.
Greening......	1—1	L-S, N	0	0	0	0	0	0	0
“	1—2	“ “	0	0	0	0	0	0	0
“	1—3	“ “	0	0	0	0	0	0	0
“	1—4	“ “	0	0	0	0	0	0	0
“	1—5	“ “	33	0	0	0	33	67	226
“	1—6	“ “	11	0	0	0	11	31	66
“	1—7	“ “	32	0	0	0	32	64	164
“	1—8	“ “	10	0	0	0	10	18	62
“	1—9	“ “	5	0	0	0	5	25	30
“	6—1	Check	*350	0	0	0	350	1,627	1,873
“	2—1	L-S, N	0	0	0	0	0	0	0
“	2—2	“ “	0	0	0	0	0	0	0
“	2—3	“ “	0	0	0	0	0	0	0
“	2—4	“ “	0	0	0	0	0	0	0
“	2—5	“ “	1	0	0	0	1	5	6
“	2—6	“ “	2	0	0	0	2	10	12
“	2—7	“ “	21	0	0	0	21	64	86
“	7—2	Check	*700	0	0	0	700	2,961	3,325
“	3—1	L-S, N	0	0	0	0	0	0	0
“	3—2	“ “	0	0	0	0	0	0	0
“	3—3	“ “	0	0	0	0	0	0	0
“	3—4	“ “	0	0	0	0	0	0	0
“	3—5	“ “	4	0	0	0	4	20	22
“	3—6	“ “	0	0	0	0	0	0	0
“	3—7	“ “	0	0	0	0	0	0	0
“	7—4	Check	*1,000	0	0	0	1,000	3,430	4,564
“	4—1	L-S, N	7	4	0	0	11	35	50
“	4—2	“ “	0	0	0	0	0	0	0
“	4—3	“ “	0	0	0	0	0	0	0
“	4—4	“ “	6	0	0	0	6	30	42
“	4—5	“ “	0	0	0	0	0	0	0
“	4—6	“ “	5	0	0	0	5	25	30
“	4—7	“ “	0	0	0	0	0	0	0
“	7—5	Check	*2,000	0	0	0	2,000	7,215	8,411

* Estimated.

Aside from the effects of the nicotine solution on the insects, another result of the spraying was the protection afforded to the foliage from apple scab. The leaves of the sprayed trees on June 21 were flattened and of a deep green color, showing comparative freedom from scab infections. The conditions of the checks at this time were, however, quite different, a goodly percentage of the foliage being yellowish, curled or otherwise deformed. Many of the leaves were also dropping, chiefly thru attacks of the fungus, altho the rosy aphis was responsible for a small amount of the defoliation.

Test 2. Orchard of C. D. Tabor, Wilson.— This test was made on a plat comprising twenty trees of the variety Baldwin and eight trees of the variety Greening, all of which are about thirty-six years of age. Lime-sulphur solution and nicotine sulphate (Black Leaf 40) was applied on May 1 and 3 respectively, when the buds on the Baldwin trees had mostly opened and the young leaves, altho compact, had extended about one-fourth inch. On the Greenings the buds were somewhat more advanced and occasional young leaves had unfolded. To insure a liberal wetting of the fruit spurs an average of twenty gallons of the spraying mixture was applied to each tree.

Infestation of the buds with aphides was not excessive, altho the young lice were rather evenly distributed over the trees in the proportion of one to eighteen of the creatures to a bud. The rosy and grain aphides were both present, the latter being, as usual in this orchard, the more abundant species. *Avenæ* developed rapidly during the month of May but *sorbi* became more abundant during early June, when its destructive work was quite apparent. Toward the middle of the month leaf and fruit clusters and young fruits on unsprayed trees were badly deformed thru attacks by increasing numbers of the insects. On June 21 the sprayed trees showed only slight attacks from the aphides, and by actual counts on twelve trees infestation by this species varied from two to forty-three centers to a tree. In the unsprayed section of the experiment the lice were more abundant, there being from one hundred five to two hundred sixteen affected fruit and leaf clusters on each tree, see Table XI. The foliage on the checks was noticeable for its lack of vigor and subsequent curling and dropping of many of the leaves. This condition was due to an early infection of apple-scab fungus which was not checked by the schedule of spraying usually followed in this orchard. The trees in the treated plat showed much less evidence of this disease, and for the most part the foliage was healthy, contrasting quite strongly with that of the unsprayed plat.

TABLE XI.— RESULTS OF SPRAYING AGAINST ROSY APHIS.

Tabor orchard.

VARIETY.	LOCATION.	Treatment.	NUMBER OF DIFFERENT SECTIONS AFFECTED.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row, Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.			
Baldwin......	1— 1	L-S, N	9	0	0	0	9	7	27
"	1— 2	" "	12	0	0	0	12	8	28
"	1— 3	" "	6	1	0	0	7	0	19
"	1— 4	" "	2	0	16	0	18	4	92
"	1— 5	" "	2	0	0	0	2	2	12
"	1— 6	" "	6	0	0	0	6	12	15
"	1— 7	" "	9	0	0	0	9	42	20
"	1— 8	" "	5	0	6	0	11	14	48
"	1— 9	" "	9	1	0	0	10	9	51
"	1—10	" "	18	0	11	0	29	52	162
"	1—11	" "	15	0	0	0	15	30	40
"	1—12	" "	39	4	0	0	43	64	142
"	2— 2	Check	103	2	0	0	105	411	622
"	2— 3	"	214	2	0	0	216	742	1,296
"	2— 4	"	193	0	0	0	193	784	1,347

Test 3. Orchard of B. S. Harwood, Appleton.— The experiment comprised a block of thirty-six Greening apples of about fifty-one years of age which have attained an unusual height and a corresponding spread of branches. An application of lime-sulphur and nicotine sulphate (Black Leaf 40) was made on April 27 and 28 at the rate of 27.7 gallons per tree.

When spraying commenced the young buds were well advanced; in some instances the terminals had pushed out about one-half inch and single leaves were spreading. Two species of lice were abundant, which were *avenæ* and *sorbi.* The infestation was not large but the insects were well distributed in the proportions of one to twenty or more to a bud. As the season advanced the numbers of aphides, as indicated by conditions of the checks, increased alarmingly during the months of May and June, causing conspicuous curling of leaves and dwarfing of fruits.

On June 21 comparatively few of the insects were seen in the sprayed plat, but in the untreated portion of the orchard the infestation ranged from three hundred to seven hundred leaf and fruit clusters to each tree, see Table XII. There were also differences in the conditions of the two plats with respect to infection of the foliage with apple scab. Besides the dropping of leaves weakened by attacks of the lice there was considerable defoliation from apple scab. The ground beneath the trees was carpeted with the fallen leaves which showed distinctly areas of infection by the fungus. The checks received the usual sprays for scab and codling moth, but these applications did not appear to prevent considerable infection of the leaves by the fungus. The remainder of the orchard was, however, unaffected to any appreciable extent by apple scab, which indicated that the delayed dormant treatment was of considerable benefit in preventing the early establishment of the disease in the foliage on these trees.

Test 4. Orchard of F. L. Backus, Olcott.— A plat of thirty-two Greening trees about twenty-seven years of age was reserved for

TABLE XII.— RESULTS OF SPRAYING AGAINST ROSY APHIS.

Harwood orchard.

VARIETY.	LOCATION.	Treatment.	NUMBER OF DIFFERENT SECTIONS AFFECTED.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row, Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.			
Greening......	1—1	L-S, N	6	1	0	0	7	12	27
"	1—2	" "	0	0	0	0	0	0	0
"	1—3	" "	6	0	0	0	6	16	32
"	1—4	" "	10	0	0	0	10	32	47
"	1—5	" "	0	0	0	0	0	0	0
"	1—6	" "	0	0	0	0	0	0	0
"	1—7	" "	3	0	0	0	3	7	11
"	1—8	" "	0	0	0	0	0	0	0
"	1—9	" "	5	0	0	0	5	22	26
"	5—2	Check	*700	0	0	0	700	2,700	2,821
"	2—1	L-S, N	0	0	0	0	0	0	0
"	2—2	" "	0	0	0	0	0	0	0
"	2—3	" "	3	0	0	0	3	2	12
"	2—4	" "	1	2	0	0	3	5	14
"	2—5	" "	2	1	0	0	3	8	12
"	2—6	" "	5	2	0	0	7	20	31
"	2—7	" "	6	1	0	0	7	23	41
"	2—8	" "	14	0	0	0	14	52	81
"	5—3	Check	*300	0	0	0	300	1,188	1,501
"	3—1	L-S, N	0	0	0	0	0	0	0
"	3—2	" "	0	0	0	0	0	0	0
"	3—3	" "	0	0	0	0	0	0	0
"	3—4	" "	0	0	0	0	0	0	0
"	3—5	" "	0	0	0	0	0	0	0
"	3—6	" "	5	11	0	0	16	5	82
"	4—1	" "	3	0	0	0	3	7	12
"	4—2	" "	2	0	0	0	2	6	5
"	4—3	" "	2	0	0	0	2	4	7
"	4—4	" "	1	0	0	0	1	4	5
"	4—5	" "	0	0	0	0	0	0	0
"	4—6	" "	14	2	0	0	16	67	62
"	4—7	" "	3	0	0	0	3	10	12
"	4—8	" "	0	0	0	0	0	0	0
"	4—9	" "	1	0	0	0	1	4	6
"	5—4	Check	*600	0	0	0	600	2,430	2,427
"	5—3	"	*700	0	0	0	700	2,627	2,943

* Estimated.

the test. These were sprayed on April 27 with lime-sulphur solution to which was added arsenate of lead and nicotine sulphate (Black Leaf 40). At this time the buds were compact but the green tissues were projecting nearly one-fourth inch beyond the bud scales. The infestation of the trees with the insects was moderate and averaged between one and eleven of the creatures to a bud. The aphides which were chiefly observed on the lower limbs were largely of the species *avenæ.* The rosy aphis was numerous enough, however, to raise a question as to the advisability of treating the entire planting, which led to the decision by the owner to spray all the orchard upon the completion of the experimental operations.

On June 27 the experimental plat was examined and the number of infested clusters on each tree was obtained by count, which showed all but eight of the sprayed trees to be entirely free from aphides and these contained only from one to four centers of infestation. On the check, situated in the same row, there were 186 infested fruit clusters. The conditions of the different trees are shown in Table XIII.

The entire orchard received the " delayed dormant " application and in addition the usual treatments of the spraying schedule. Few differences were noticed between the sprayed and unsprayed trees on June 21, but the check appeared slightly less thrifty and the leaves showed a somewhat larger number of scab areas than the other trees in the orchard. The fruit when harvested was somewhat scabby, but on the whole there was, as regards the quality of the fruit in this respect, little difference between the check and the sprayed trees.

Test 5. Orchard of H. H. Freeman, Kent.— This entire planting is annually treated with a " delayed dormant " spray. There was reserved, however, for the test a block of thirty-eight Greening trees averaging between thirty-one and thirty-six years of age, and of these six trees were reserved as checks.

The plat was sprayed on April 22 with lime-sulphur and nicotine sulphate (Black Leaf 40) in the usual proportions. At this time only a few insects, which were chiefly oat aphides, were on the buds. Immediately after the spraying was completed an examination of the trees indicated that a large percentage of the young lice had been destroyed. As the season advanced few differences were noted in the conditions of the sprayed and unsprayed plats and on June 21 while the sprayed trees were either free from aphides or had from one to four centers of infestation, the count on the checks showed only three injured clusters.

Table XIII.— Results of Spraying Against Rosy Aphis.

Backus orchard.

Variety.	Location.	Treatment.	Number of Different Sections Affected.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row, Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.			
Greening......	5— 1	L-S, N	0	0	0	0	0	0	0
"	5— 2	" "	0	0	0	0	0	0	0
"	5— 3	" "	2	0	0	0	2	5	4
"	5— 4	" "	0	0	0	0	0	0	0
"	5— 5	" "	0	0	0	0	0	0	0
"	5— 6	" "	0	0	0	0	0	0	0
"	5— 7	" "	0	0	0	0	0	0	0
"	5— 8	" "	0	0	0	0	0	0	0
"	5— 9	" "	0	0	0	0	0	0	0
"	5—10	" "	4	0	0	0	4	8	8
"	5—11	" "	1	0	0	0	1	2	3
"	5—12	" "	1	0	0	0	1	1	2
"	5—13	" "	0	0	0	0	0	0	0
"	5—14	" "	2	0	0	0	2	6	6
"	5—15	" "	0	0	0	0	0	0	0
"	5—16	Check	186	2	0	0	188	476	857
"	6— 1	L-S, N	0	0	0	0	0	0	0
"	6— 2	" "	0	0	0	0	0	0	0
"	6— 3	" "	0	0	0	0	0	0	0
"	6— 4	" "	2	0	0	0	2	3	5
"	6— 5	" "	0	0	0	0	0	0	0
"	6— 6	" "	0	0	0	0	0	0	0
"	6— 7	" "	0	0	0	0	0	0	0
"	6— 8	" "	0	0	0	0	0	0	0
"	6— 9	" "	0	0	0	0	0	0	0
"	6—10	" "	0	0	0	0	0	0	0
"	6—11	" "	0	0	0	0	0	0	0
"	6—12	" "	3	0	0	0	3	10	17
"	6—13	" "	0	0	0	0	0	0	0
"	6—14	" "	1	0	0	0	1	2	4
"	6—15	" "	0	0	0	0	0	0	0
"	6—16	" "	0	0	0	0	0	0	0

Test 6. Orchard of John Larwood, Albion.— This is an orchard of the variety Baldwin which each year has always exhibited varying amounts of injury from aphides, especially the rosy aphis. For the test eighteen trees were selected which were between thirty-six and forty-one years of age. The remainder of the planting received no treatment and served as a check on the experiment. Spraying commenced on April 28 just as the green ends of the buds were showing and before the young leaves had unfolded. The spraying mixture was the combination used in the preceding tests and each tree received about eleven gallons.

When spraying commenced a moderate number of *avenæ* and *sorbi* were detected on the buds which were rather evenly distributed, especially on the lower limbs of the trees. Altho the conditions during this season were not especially favorable to the development of large numbers of the insects, the unsprayed section of the planting during early June showed quite a little curling and distortion of leaf and fruit clusters by the rosy aphis. On June 20 practically no infestation was found on the sprayed trees, while none of the checks were free from injured fruit clusters as shown in Table XIV.

TABLE XIV.— RESULTS OF SPRAYING AGAINST ROSY APHIS.

Larwood orchard.

VARIETY.	LOCATION.	Treatment.	NUMBER OF DIFFERENT SECTIONS AFFECTED.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row, Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters	Water sprouts.			
Baldwin......	7— 1	L-S, N	0	0	0	0	0	0	0
"	7— 2	" "	0	0	0	0	0	0	0
"	7— 3	" "	2	0	1	0	3	7	4
"	7— 4	" "	0	0	0	0	0	0	0
"	7— 5	" "	10	0	1	0	11	31	22
"	7— 6	" "	0	0	0	0	0	0	0
"	7— 7	" "	0	0	0	0	0	0	0
"	7— 8	" "	0	0	0	0	0	0	0
"	7— 9	" "	0	0	0	0	0	0	0
"	7—10	" "	0	0	0	0	0	0	0
"	7—11	" "	0	0	0	0	0	0	0
"	7—12	" "	0	0	0	0	0	0	0
"	7—13	" "	0	0	0	1	1	0	6
"	7—14	" "	1	0	0	0	1	0	5
"	7—15	" "	3	0	0	0	3	13	5
"	8— 6	Check	42	0	21	0	63	89	305
"	9— 7	"	17	0	3	0	20	65	50
"	9— 5	"	9	0	4	0	13	21	40

Mention should also be made of the fact that this orchard received all the usual sprayings except the " delayed dormant " application which was made only to the experimental plat. In view of this difference in treatment it is interesting to note that on June 20 the trees in the test plat possessed healthy foliage, the leaves being of a dark green color and largely free from distortions. The unsprayed trees, however, were sickly in appearance, the leaves showing a tendency to curl and become yellow and finally to drop. Subsequent to this partial defoliation there was little difference in the conditions of the trees in either of the plats with respect to this disease, as the regular treatments with lime-sulphur protected the new foliage from further infection. The crop of apples was small but the greater bulk of the fruit was obtained from the trees that received the delayed dormant treatment to control the rosy aphis.

Test 7. Orchard of John Beckwith, Lyndonville.— There was sprayed in this orchard a block of fifty Baldwin trees about forty-one years of age. These trees have been for a number of years more or less seriously infested with the rosy aphis and the owner has, during the last few years, been obliged to take special precautions against the pests.

The trees were sprayed on April 20 as the buds were breaking and the young lice were clustered in small numbers on the opening buds. An average of fifteen gallons of nicotine sulphate (Black Leaf 40) solution at standard dilution was applied to each tree. While in the balance of the orchard and on the checks a sizable infestation developed, on June 20 only an occasional single cluster was discovered on the sprayed plat. The results of counts of clusters are given in Table XV.

TABLE XV.— RESULTS OF SPRAYING AGAINST ROSY APHIS.

Beckwith orchard.

VARIETY.	LOCATION.	Treatment.	NUMBER OF DIFFERENT SECTIONS AFFECTED.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row, Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.			
Baldwin......	1— 1	Check	11	4	30	0	45	20	144
“	1— 2	L-S, N	0	0	0	0	0	0	0
“	1— 3	“ “	0	0	0	0	0	0	0
“	1— 4	“ “	0	0	0	0	0	0	0
“	1— 5	“ “	0	0	0	0	0	0	0
“	1— 7	“ “	0	0	0	0	0	0	0
“	1— 8	“ “	0	0	0	0	0	0	0
“	1— 9	“ “	0	0	0	0	0	0	0
“	1—10	“ “	0	0	0	0	0	0	0
“	1—11	“ “	0	0	0	0	0	0	0
“	1—12	“ “	0	0	0	0	0	0	0
Greening......	1—13	“ “	0	0	0	0	0	0	0
Baldwin......	1—14	“ “	0	0	0	0	0	0	0
“	2— 1	Check	33	0	32	0	65	93	245
“	2— 2	L-S, N	0	0	5	0	5	0	7
“	2— 3	“ “	0	0	0	0	0	0	0
“	2— 4	“ “	4	0	1	0	5	6	16
“	2— 5	“ “	0	0	0	0	0	0	0
“	2— 6	“ “	0	0	0	0	0	0	0
“	2— 7	“ “	0	0	0	0	0	0	0
“	2— 8	“ “	2	0	0	0	2	3	2
“	2— 9	“ “	0	0	0	0	0	0	0
“	2—10	“ “	0	0	0	0	0	0	0
“	2—11	“ “	0	0	0	0	0	0	0
“	2—12	“ “	0	0	0	0	0	0	0
“	2—13	“ “	0	0	0	0	0	0	0
“	2—14	“ “	0	0	0	0	0	0	0

Test 8. Orchard of H. B. Gibson, Albion.— This is a planting of mixed varieties of such kinds as Baldwin, Greening, Twenty Ounce and Tolman Sweet. The trees are about thirty-seven years of age and have not received attention as regards plant lice except in a section treated during the spring of 1915. *Avenæ* and *sorbi* were both observed as the buds opened. Of these the oat aphis was the more numerous at the time the trees were sprayed on April 27. Breeding by the insects at the beginning of the season was not excessive, and on May 9 specimens of aphides were found with difficulty. However, beginning with this date and continuing through the month of June the numbers of the rosy aphis increased rapidly and on June 21 the unsprayed portion of the orchard showed considerable harm by the insects. In comparison the sprayed trees had few aphides and in general the condition of this block as regards rosy aphis was excellent, see Table XVI. When the fruit was picked the trees which received the " delayed dormant " application yielded proportionately more apples than adjacent trees not so treated, which was largely the result of the joint control of the rosy aphis and apple scab.

Table XVI.— Results of Spraying Against Rosy Aphis.

Gibson orchard.

Variety.	Location.	Treatment.	Number of Different Sections Affected.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row, Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.			
Greening......	8—1	L-S, N	1	0	0	0	1	3	6
"	8—3	" "	8	0	4	0	12	41	40
"	8—2	Check	112	0	47	2	161	250	394
Baldwin......	6—1	L-S, N	2	0	1	0	3	7	15
"	6—2	" "	0	0	0	0	0	0	0
"	6—3	" "	5	0	2	0	7	15	12
"	6—4	" "	0	0	0	0	0	0	0
"	6—5	" "	2	0	0	1	3	5	8
"	6—6	" "	0	0	0	0	0	0	0
Tolman Sweet..	6—7	" "	0	0	0	0	0	0	0
" " .	6—8	" "	1	0	3	0	4	2	22
Baldwin......	5—1	Check	63	0	1	1	65	259	280
Twenty Oz....	7—1	L-S, N	0	0	0	0	0	0	0
"	7—2	" "	1	0	3	0	4	1	20
"	7—3	" "	0	0	0	0	0	0	0
"	7—4	" "	0	0	0	0	0	0	0
"	7—5	" "	0	0	0	3	3	0	14
"	7—6	" "	0	0	0	0	0	0	0
"	7—7	" "	1	1	2	2	6	1	22
"	7—8	" "	1	1	0	0	2	0	8

Test 9. Orchard of G. E. Snyder, Albion.— Twenty-five Greening trees were selected for the test in this orchard, while the remainder of the planting served as a check on the treatment to combat the aphides. The plat was sprayed on April 28, using lime-sulphur and nicotine sulphate (Black Leaf 40) at the rate of ten gallons to each tree. The aphides were not numerous when spraying was attempted and only one to eleven individuals were seen on a bud, which were chiefly *avenæ* and *sorbi.* The multiplication of the insects at first was not rapid and on May 11 only scattering colonies of either species could be found. Subsequent to this date the insects increased rapidly in numbers, so that by June 21 the unsprayed block was so badly injured that it was not possible to make accurate counts of the injured apples. Associated with the destructive work of the aphides there was considerable infection of the foliage by apple scab which resulted in a large percentage of the leaves becoming yellow and dropping so that the ground beneath the trees was covered with them. The sprayed trees sustained some damage from apple scab, but there was practically no defoliation. The conditions with respect to rosy aphis are shown in Table XVII.

Table XVII.— Results of Spraying Against Rosy Aphis.

Snyder orchard.

Variety.	Location.	Treatment.	Number of Different Sections Affected.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row, Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.			
Greening......	2—1	L-S, N	2	0	9	0	11	4	17
"	2—2	" "	5	0	1	0	6	4	18
"	2—3	" "	0	0	0	0	0	0	0
"	2—4	" "	19	2	11	0	32	55	91
"	2—5	" "	1	0	1	0	2	1	6
"	3—1	" "	8	0	9	0	17	10	68
"	3—2	" "	2	0	0	0	2	2	3
"	3—3	" "	4	0	0	0	4	7	8
"	3—4	" "	2	0	0	1	3	2	6
"	3—5	" "	1	0	0	0	1	1	3
"	1—1	" "	3	0	0	0	3	3	5
"	1—2	" "	4	0	1	0	5	10	13
"	1—3	" "	1	0	0	0	1	1	3
"	1—5	" "	1	0	1	0	2	1	6
"	1—6	*Check							
"	1—7	*Check							
"	2—7	*Check							

* Severe infestation by aphis and extensive defoliation by scab prevented accurate accounts.

Test 10. Orchard of Mrs. Ida Lafler, Albion.— In this experiment thirty-five trees were selected for treatment. These were Greenings about 35–40 years old which had been well cared for and have been very productive. An application of the lime-sulphur, lead arsenate, nicotine sulphate (Black Leaf 40) combination was made on April 27, using 17 gallons per tree. The infestation of aphides was not large but on many buds as high as twenty-five or thirty young nymphs were counted. On May 11 *sorbi* was scarce, but more specimens of this species were observed on the untreated trees than on those sprayed. On June 20 the infestation on twenty-two trees was counted, six of which were checks. Nine of the sprayed trees were entirely free from evidences of any injury, and others had a varying number of centers of infestation which were never more than seven. The unsprayed trees were in every case much more infested, as may be observed in Table XVIII.

TABLE XVIII.— RESULTS OF SPRAYING AGAINST ROSY APHIS.

Lafler orchard.

VARIETY.	LOCATION.	Treatment.	NUMBER OF DIFFERENT SECTIONS AFFECTED.				Centers of infestation.	Fruits injured.	Leaves curled.
	Row, Tree.		Fruit clusters.	Terminal shoots.	Leaf clusters.	Water sprouts.			
Greening......	5—1	L-S, N	0	0	0	0	0	0	0
“	5—2	“ “	0	0	0	0	0	0	0
“	5—3	“ “	0	0	0	0	0	0	0
“	5—4	“ “	0	0	0	0	0	0	0
“	6—1	“ “	4	0	0	0	4	0	20
“	6—2	“ “	4	0	3	0	7	8	32
“	5—3	“ “	0	0	6	0	6	0	21
“	6—4	“ “	2	0	5	0	7	5	19
“	7—1	“ “	1	0	3	0	4	3	14
“	7—2	“ “	0	0	2	0	2	0	4
“	7—3	“ “	0	0	0	0	0	0	0
“	7—4	“ “	0	0	0	0	0	0	0
“	8—1	“ “	1	0	4	0	5	0	19
“	8—2	“ “	0	0	0	0	0	0	0
“	8—3	“ “	0	0	0	0	0	0	0
“	8—4	“ “	0	0	0	0	0	0	0
“	10—1	Check	18	0	21	0	39	39	134
“	10—2	“	38	0	21	0	59	56	201
“	11—3	“	39	0	57	0	96	103	449
“	12—4	“	55	0	46	0	101	124	360
“	12—5	“	60	0	30	0	90	122	393
“	12—6	“	49	0	23	0	72	149	313

Reference has frequently been made to the protection from apple scab afforded by the delayed dormant treatment to the foliage and in none of the experiments were these results more marked than in this orchard. By June 21 the unsprayed trees were being rapidly defoliated, and coupled with this there was considerable dropping of the young fruits. The ground beneath the trees was literally a carpet of leaves. Here again the treated trees presented superior conditions; for, while there was more or less infection of the foliage, dropping of the leaves was little in evidence. When the crop was picked more apples proportionately were gathered from the sprayed plot than from the unsprayed trees in the orchard.

Test 11. Orchard of Lynn Burrows, Albion.— This planting comprises nearly one hundred Wealthy and Duchess apples of about 13 years of age. Spraying commenced on April 29 and was completed the following day. Seventy-five trees were sprayed, using one hundred ninety gallons of lime-sulphur, lead arsenate and Black Leaf 40. The oat aphis was most numerous in this planting, with which there were occasional specimens of the rosy aphis. Development of the insects was slow as indicated by a record of May 11 when scattering colonies of *avenæ* were found on the unsprayed section. On June 21, however, only an occasional cluster was observed on either plat, for which reason no conclusions can be drawn from the work in this planting.

Test 12. Orchard of John Beckwith, Demster.— In this planting a row of seventeen Greening trees about thirty-five years of age was reserved for the test. The rosy aphis has been very destructive for a number of years in this orchard, causing considerable annual reduction in the crop of apples. On May 3 the plat was sprayed with lime-sulphur, arsenate of lead and Black Leaf 40 in the usual proportions, using on an average about 17 gallons to each tree. Two trees were left as a check, one of which was a Greening. The rosy aphis was not abundant on the trees at the time of spraying, but as the season advanced the insects rapidly increased in numbers so that during June the checks were abundantly infested. On June 26 the Greening check showed ninety-eight centers of infestation and the Tolman Sweet one hundred and one colonies. On the sprayed portion of the plat no injured clusters were observed and the balance of the orchard, which was sprayed at the same time, was entirely free from rosy aphis. On the trees of the variety Tolman Sweet, which were sprayed by the owner, no infested clusters were found, which it will be observed is in strong contrast to the condition of the check tree of the same variety in this experiment.

THE DELAYED DORMANT APPLICATION IN THE SPRAYING SCHEDULE.

One of the important applications in the approved spraying schedule for apple orchards is a treatment with lime-sulphur solution at winter strength in the spring when the buds are dormant or are showing green tissues at the ends. This is commonly designated the " dormant application " and it is usually made for the almost exclusive purpose of combating the San Jose scale and blister mite. It undoubtedly exerts other beneficial results, but what destructive organisms are affected and to what extent there is little definite knowledge by either plant pathologists or entomologists.

As has been previously indicated the foregoing application is of little or no value against the rosy aphis since it is made at a time when the insect is in the egg stage and, as far as this species is concerned, lime-sulphur does not function to any important extent as an ovicide. Growers who are troubled with the aphis are therefore confronted with the problem of making a special treatment which, on account of the capriciousness of the insect, expense and pressure of other work is, under average farm conditions, impracticable. One possible solution to this dilemma is not to make the first application with lime-sulphur so early in the spring as is usually done, but rather to postpone the operation until the eggs of the rosy aphis have hatched, when nicotine solution should be combined with the lime-sulphur mixture to destroy the young nymphs of this insect. To be effective the spraying with this combination should not be done until the leaves of the more advanced blossom buds are projecting as much as one-fourth to half an inch. For ease of reference and to avoid confusion it is proposed to designate the treatment when buds are in this advanced stage of growth as the " delayed dormant application," a term which has already been used by several writers. This change in the time of making the first treatment has certain advantages and some disadvantages which growers should clearly understand. These may be briefly summarized as follows:

ADVANTAGES.

1. Obviates necessity of special treatment for rosy aphis.
2. Application of strong mixtures of lime-sulphur for San Jose scale affords some protection from early infections from apple scab.[1]

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[1] The Oregon Experiment Station (Rept. of Hood River Branch for 1914–15) reports that in two experiments there was on the average 12 per ct. more scab-free

3. Combination of nicotine solution with lime-sulphur controls not only the rosy aphis but other aphides and insects.
4. Addition of arsenate of lead to lime-sulphur is destructive to various early-appearing leaf-eating caterpillars as bud moth, casebearers, etc.
5. Thoroness of treatment required for the control of San Jose scale is likely to result in more effective work against other pests than when special sprayings are made.

Disadvantages.

1. Increasing pressure of other farm operations as the season advances.
2. With development of high temperatures favorable for rapid unfolding of buds, danger of injury to leaves by late spraying with lime-sulphur at winter strength. Fear of damaging foliage may lead to abandonment of treatment, which would leave orchard with insufficient protection against the San Jose scale.
3. Possible unsatisfactory protection against the blister-mite, owing to the lateness of the application.

On the whole we have found in our experience that the advantages from the delayed dormant treatment outweigh the disadvantages, altho some of the latter can by no means be ignored. The proper adjustment of the various activities of the farm to insure adequate treatment of apple orchards are problems that constantly beset most growers. This consideration presents no new difficulty either in kind or degree and, as shown by auxiliary experiments with commercial growers, is capable of practical solution. The danger of leaf injury should be noted. It is true that damages may result from drenching of the trees with lime-sulphur solution at " winter " strength. Usually these are not important, and such as occur are quickly obscured by the unfolding of the new foliage. The effect of the omission of the ordinary dormant treatment on the blister-mite has not been indicated as far as any negative results are concerned. This is of itself significant. The pest has not shown itself in any of the plats on the Experiment Station grounds or in ten or more large apple orchards under our control in western New York that have been given the delayed dormant treatment for the past two years. Until there is evidence to the contrary little attention will be given to the blister-mite in our experimental activities, since an efficient control of the rosy aphis is a more important consideration.

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fruit from this application. In most of the experiments conducted by this Station during 1916 there was noticeable protection to apple foliage as a result of a late spraying with lime-sulphur at winter strength.

PLAN FOR A SPRAYING TEST.

While experience shows that the protection of apple orchards from the rosy aphis is not difficult of demonstration, present indications are that many orchardists will not modify at a single stroke their spraying practices to obtain better protection from this pest. Their failure to do so is attributable to various reasons. First of all, the insect may vary greatly in importance in individual orchards from year to year. During most seasons the extent of damage by the aphis is not large, and spraying to control it would probably cost as much as the fruit that is threatened is worth. While the majority of plantings sustain great damage in occasional years, the orchard that is annually subject to extreme injury is exceptional. Such experiences tend to discourage systematic spraying to control this pest. Opposed to this position is the outstanding fact that if one does not spray his trees systematically he is likely to sustain severe losses during certain seasons; and the value of the shrinkage in fruit yields may entail a greater financial loss than is expended in spraying for several years. Another phase of the problem should also be noted. Most growers who undertake to spray for the rosy aphis fail in their efforts because they fall short of the standards to do effective work. Literally speaking, the trees need to be drenched with the spraying mixture so that not a single bud or at the most only a few buds escape treatment. As a rule the grower does not do thoro work, largely because he applies too little of the spraying mixture and does not exercise enough pains to wet all of the buds.

In view of these facts we suggest to orchardists who are not certain of the practicability of spraying for the rosy aphis that they undertake a spraying experiment for a period of several years, rather than attempt at the outset to treat their entire plantings. In making a test select a small portion of an orchard, as one or two short rows or a plat of ten to twenty trees. For the sake of economy in time and materials the trees should not be too large, preferably about twenty years of age and of a susceptible variety, as Greening, Rome, Northern Spy or Twenty Ounce. For the spraying mixture apply either lime-sulphur and nicotine solution or soap and nicotine solution under the conditions as recommended by this Station. The test is both simple and inexpensive. If properly carried out no other method is so well calculated to direct an orchardist along proper lines, since it will enable him to determine to what extent the rosy aphis reduces his yields and also to ascertain if the protection obtained pays for the additional expense of spraying materials to combat the insect. The results from such a test ought to demonstrate to the grower the feasibility of undertaking larger operations as a regular procedure in the upkeep of his apple plantings.

THE RADISH MAGGOT.*

P. J. PARROTT AND H. GLASGOW.

SUMMARY.

The radish or cabbage maggot is a common pest of radishes. The insect varies in importance from year to year. During some seasons it is very destructive, reducing the yield and quality of marketable roots.

Injuries to radishes occur during the latter part of May and early June. As grown in most home gardens the time of maturing of roots coincides with the period of egg deposition and development of larvæ of first brood. Plantings which attain marketable size as first-brood larvæ are maturing usually sustain the most serious damage.

Experiments during the past four years to determine the influence of time of planting upon the numbers of insects and extent of injury showed, in 1914, severe infestation in plantings of radishes maturing about June 9; for 1915 and 1916, considerable infestation in plants harvested about June 1 and June 15 respectively; and, for 1917, slight injuries only during June. In general, radishes pulled before the latter part of May have been free from important injuries.

Screening of radish beds is an efficient method of affording protection from maggot injury. Under normal seasonal conditions plants raised in screened frames grow more rapidly and have generally been superior in size, succulence and tenderness to those produced in open beds. In 1917 when temperature and precipitation during growing months were abnormal only slight differences existed in the conditions of plants produced by the two methods.

Practical measures for production of radishes that are largely exempt from insect attack are early sowing and growing of plants in frames screened with cheesecloth.

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* Reprint of Bulletin No. 442, November, 1917.

INTRODUCTION.

The radish or cabbage maggot, *Phorbia brassicæ* Bouché, is of three-fold importance: (1) The insect dwarfs and destroys early cabbage; (2) it attacks seedlings of late cabbage, resulting during some seasons in a serious shrinkage in the output of seed-beds; and (3) it causes maggoty radishes and, in addition to reducing the quality of the roots, may either stunt or destroy the plants. The destructive activities of the pest with reference to the culture of early and late cabbage are discussed in Bulletin No. 419 of this Station. In the study here presented attention is directed to the injurious work of the insect on radishes and to experiments with screening as a means of protecting radish beds.

THE INSECT AND ITS RELATIONS TO RADISH CULTURE.

The adult (Plate XXXI, fig. 4) of the radish maggot is a fly, resembling the common house fly, but smaller, measuring about one-fourth of an inch in length and with a narrower body and proportionately larger wings. The thorax is ash gray in color with three distinct longitudinal lines on dorsum. The body is covered with numerous hairs or bristles. The legs are black and bristly, with a tuft of short bristles at the base of the posterior femur, which serves to distinguish the males of this species from males of closely allied forms. The eggs (Plate XXXI, fig. 1) are white, glistening and marked with irregular longitudinal furrows. They measure about one twenty-fifth of an inch in length. The larva or maggot (Plate XXXI, fig. 2) is a white, fleshy creature, at first cylindrical and tapering anteriorly, but later, toward the end of the feeding period, with middle segments much enlarged. The third stage of the insect from the egg is a puparium (Plate XXXI, fig. 3). This is a brownish body, elongate ovate in form and bluntly rounded at the ends. Large individuals measure about one-fourth of an inch in length. The outer protective covering is formed by the hardening and contraction of the integument of the third larval instar, and within this the pupa is formed. It is the inactive stage of the species and comparable to the chrysalis of moths and butterflies.

Studies on the life history of the insect at this Station have shown that the first of the adults or flies to emerge in the spring make their appearance during the first two weeks of May, the actual time of their emergence depending somewhat on the weather. The flies apparently do not appear simultaneously, as in one observation they emerged for a considerable period, from May 9 to June 14 or about thirty-six days. Within a few days after their escape from the ground they commence to deposit eggs. In this operation the female generally alights on the outer margin of a leaf. This she examines, running quickly from one area of inspection to another. She then crawls down the stalk of the plant to the ground, and upon finding a crevice or chink she lays her eggs. Oviposition is usually very active during the latter part of May and during early June. The eggs hatch within three to five days. The larvæ or maggots attack the roots, and it is this stage which is responsible for injuries to radishes, early cabbage and seedlings of late-maturing varieties of cabbage. The creature may be found in hidden tunnels, penetrating to varying depths, or in more or less open grooves along the surface of the roots. The laceration of the tissues is often accompanied by decay of the injured areas. The destructive work of the maggot is generally most evident in June, although the time of appearance of adults and the period of egg-laying and damage by the insect are hastened by warm weather during April and May. Upon maturity the larva generally abandons its host and tunnels the ground in the immediate vicinity of the plants, when it transforms to the pupal stage. A large percentage of the puparia are within three inches of the injured plant and in the first three inches of soil. Occasionally puparia may be found in the plants themselves when these have a tuber-like root, as radishes or turnips. The pupal stage lasts from twelve to eighteen days, although it may be prolonged for an indefinite period of several months. When favorable conditions exist there are at least three broods and perhaps a partial fourth brood.

Mention has just been made of the number of broods. It should be stated in this connection that, while with a proper selection of varieties radishes may be grown in this latitude over an extended season, yet in actual practice the culture of the radish in open beds out of doors, in the region of Geneva, is limited largely to a short period during the months of April, May and June. As a rule, comparatively few radishes are grown much later than this period as the plants suffer from drought and heat incident to the summer.

CHART 1.— RELATION OF TIME FOR GROWING RADISHES TO DATES OF APPEARANCE OF THE MAGGOT IN ITS VARIOUS STAGES.

Time of planting and growth period of radishes.	Date.	Time of appearance of maggot.
Time of planting seed Growth period of radishes	April 15	
	“ 30	
	May 1	
	“ 2	
	“ 3	
	“ 4	
	“ 5	
	“ 6	Radish-maggot flies very numerous.
	“ 7	
	“ 8	
	“ 9	
	“ 10	
	“ 11	
	“ 12	
	“ 13	
	“ 14	
	“ 15	
	“ 16	
	“ 17	
	“ 18	
	“ 19	Eggs of flies in greatest numbers and first appearance of maggots of first brood.
	“ 20	
	“ 21	
	“ 22	
	“ 23	
	“ 24	
	“ 25	
	“ 26	
	“ 27	
	“ 28	
	“ 29	
	“ 30	
	“ 31	
	June 1	
	“ 2	
	“ 3	
	“ 4	
	“ 5	
	“ 6	
	“ 7	
	“ 8	
	“ 9	Maggots full grown and period of greatest injury to radishes.
	“ 10	
	“ 11	
	“ 12	
	“ 13	
	“ 14	
	“ 15	
	“ 16	
	“ 17	
	“ 18	
	“ 19	
	“ 20	

While the behavior of the insect in radish plantings has not heretofore been carefully considered, the life history of the pest was worked out by Schoene [1] in his study of the insect in cabbage seedling beds. From his study the activities of the first brood in radish plantings are approximately as shown in Chart 1.

STUDIES ON BEHAVIOR OF INSECT IN RADISH BEDS.

Under this heading there are presented the results of a series of experiments which were designed to obtain more specific data than heretofore available as to the period of injuries to radishes by maggots and the relative susceptibility of early and late sowings to attack. As one-half of each sowing was covered with cheesecloth, the tests also furnished opportunities to determine the influence of screening in preventing damages by maggots and flea beetles as well as on the growth and quality of the roots. The details of the experimental operations for each year are considered separately, and then the principal results are summarized and discussed in regard to their bearing on radish culture.

EXPERIMENTS DURING 1914.

For this season's work, two plats, each one hundred feet long and twelve feet wide, were laid out and top-dressed with a liberal application of a mixed fertilizer composed of sodium nitrate, 25 parts; acid phosphate, 87½ parts; and muriate of potash, 25 parts. The soil was harrowed and reduced to a fine state of tilth. On May 9 the seed was sown with a garden drill at the rate of three seeds to each two inches in rows six inches apart. As seedlings were making their appearance on May 15 one of the beds was screened with a cheesecloth sheet. Flea beetles (*Phyllotreta vittata* Fab.) were very abundant on May 23 in the unscreened bed and soon caused marked injury to the seedlings. At this date a few adults of the radish maggot were observed but the species was apparently not very numerous. On May 22 and 23 both beds were weeded and thinned, leaving the plants about two inches apart. Thruout the remainder of the month of May the uncovered planting was constantly subject to attacks by large numbers of flea beetles and there were also evidences of a small amount of injury to the plants in the screened bed.

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[1] Schoene, W. J. The Cabbage Maggot: Its Biology and Control. N. Y. Agr. Exp. Sta. Bul. 419. 1916.

On June 5, when the radishes in the protected plat were of suitable size for marketing and the unscreened planting showed abundant infestation from maggots, the roots were pulled. The yields and weights of the two plats are given in Table I.

TABLE I.— YIELDS OF SCREENED AND OPEN RADISH BEDS DURING 1914.
(Counts and weighings based on rows of 100 feet in length.)

NO. OF ROW AND TREATMENT.	Plants.	Weight of plants.		Weight of roots.		Market-able roots.	Weight of market-able roots.	
	No.	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*	*No.*	*Lbs.*	*ozs.*
Row 1, screened, thinned	506	9	9	5	4	332	4	8
Row 2, screened, thinned	332	6	14	3	15	247	3	6
Row 3, screened, thinned	324	6	4	3	10	191	2	10
Row 4, screened, thinned	347	9	3	4	7	197	3	5
Row 5, screened, not thinned	518	8	6	4	7	251	3	1
Row 6, screened, not thinned	518	9	12	5	10	265	4	3
Row 7, screened, not thinned	501	9	0	5	0	224	3	9
Row 8, screened, not thinned	605	8	12	4	9	182	2	7
Row 1, check, thinned	496	4	0	1	6	110	0	10
Row 2, check, thinned	240	1	14	1	2	83	0	11
Row 3, check, thinned	245	2	8	1	1	68	0	8
Row 4, check, thinned	230	2	9	1	1	68	0	8
Row 5, check, not thinned	532	5	9	2	6	64	0	12
Row 6, check, not thinned	478	5	1	2	8	64	0	9

SUMMARY.

	Plants.	Weight of plants.		Weight of roots.		Market-able roots.	Weight of market-able roots.	
	No.	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*	*No.*	*Lbs.*	*ozs.*
Screened plat, thinned	377	8	0	4	5	242	3	7
Screened plat, not thinned	536	9	0	4	15	231	3	5
Check, thinned	303	2	12	1	3	82	0	9
Check, not thinned	505	5	5	2	7	64	0	11
Average per 100 feet screened	456	8	8	4	10	236	3	6
Average per 100 feet check	370	3	9	1	9	76		10

EXPERIMENTS DURING 1915.

The methods of preparing the plats and sowing the seed were the same as practised in the preceding test. The dates for the different sowings were Plat I, March 15; Plat II, March 22; Plat III, April 1; Plat IV, April 5; Plat V, April 13; and Plat VI, April 14.

With each plat one-half the bed was screened immediately after the planting of the seed, while the unprotected portion of the bed served as a check. A record was kept of the daily temperatures during the growing period of the radishes, which is as follows:

TABLE II.— RECORD OF TEMPERATURES DURING EXPERIMENTS OF 1915.

DATE.	SCREENED PLATS.		UNSCREENED PLATS.		DATE.	SCREENED PLATS.		UNSCREENED PLATS.	
	Min.	Max.	Min.	Max.		Min.	Max.	Min.	Max.
	Degs. F.	*Degs. F.*	*Degs. F.*	*Degs. F.*		*Degs. F.*	*Degs. F.*	*Degs. F.*	*Degs. F.*
Mar. 29....	13	65	......	......	May 1....	45	80	40	72
30....	11	46	......	......	2....	44	59	41	56
31....	18	51	......	......	3....	32	64	28	61
April 1....	24	41	......	......	4....	33	66	28	61
2....	27	57	......	......	5....	42	63	39	58
3....	19	49	......	......	6....	41	67	38	62
4....	21	49	21	40	7....	41	83	37	75
5....	18	56	17	47	8....	49	75	45	73
6....	38	50	36	51	9....	46	85	42	79
7....	39	58	36	58	10....	32	54	26	52
8....	36	62	33	54	11....	43	75	40	74
9....	34	78	28	74	12....	47	91	43	63
10....	48	84	46	82	13....	52	86	48	84
11....	41	85	37	82	14....	36	62	34	58
12....	40	76	34	73	15....	34	74	32	61
13....	32	52	28	49	16....	33	72	30	63
14....	22	58	18	47	17....	42	50	39	50
15....	22	68	18	53	18....	33	55	30	49
16....	27	76	22	61	19....	36	64	34	53
17....	31	58	29	57	20....	35	74	31	73
18....	30	63	24	54	21....	48	84	44	75
19....	49	80	46	69	22....	50	66	47	69
20....	46	88	44	81	23....	48	79	39	74
21....	27	80	25	70	24....	39	80	38	61
22....	29	79	24	64					
23....	48	70	46	69					
24....	45	82	42	74	Total temperatures.	2,136	4,044	1,843	3,404
25....	56	95	52	90					
26....	54	105	50	93	Average per day......	37.48	70.94	36.13	66.74
27....	60	96	57	90					
28....	53	102	49	95					
29....	54	98	50	92					
30....	43	79	38	79	Average daily difference.	Min. 1.35		Max. 4.20	

During the latter portion of April flea beetles became very numerous, causing considerable damage to the leaves of the unscreened plants. They were also quite abundant in the screened beds but the evidences of their attacks were much less conspicuous since the

Table III.— Yields of Screened and Open Radish Beds during 1915.
(Counts and weighings based on rows of 100 feet in length.)

Date of Sowing.	Screened.								Unscreened.						
	Date of pulling.	Plants.	Weight of plants.		Weight of roots.		Weight of leaves.		Plants.	Weight of plants.		Weight of roots.		Weight of leaves.	
		No.	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*	*No.*	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*
Mar. 15	May 19	710	11	6	8	7	2	15	323	2	11	1	1	1	10
Mar. 22	May 19	1,092	12	4	6	4	6	0	432	2	12	1	0	1	12
April 1	May 24	1,000	16	12	9	12	7	0	680	7	4	4	4	3	0
April 5	May 24	1,004	12	4	7	0	5	4	665	7	4	3	12	3	8
April 13	May 24	1,007	9	12	5	4	4	8	520	2	8	1	0	1	8
					No. market-able roots.		Plat VI, Rows 1, 2, 3, 4. Weight market-able roots.					No. market-able roots		Weight marketable roots	
April 14	May 29	485	5	7	225		3	0	661	4	0	109		0	12
April 14	May 29	512	5	4	185		2	4	640	3	0	88		0	12
April 14	May 29	528	6	9	244		3	8	621	4	8	167		1	8
April 14	May 29	549	6	0	197		2	8	575	2	7	76		0	8

Summary.

	Plants.	Total weight.	Roots marketable.	Weight of marketable roots.
	No.	*Lbs.*	*No.*	*Lbs.*
Average per 100 feet screened (Plat VI)	519	5.8	213	2.8
Average per 100 feet unscreened (Plat VI)	624	3.5	110	0.8

plants were growing rapidly. In the open beds lack of moisture and incrustation of soil, in addition to the attacks of flea beetles, greatly retarded the growth of the plants. Until about May 18 the differences in the screened and unscreened plats were largely due to the effects of the cheesecloth in modifying their physical environment, principally as regards temperature and the moisture content of soil. Thereafter flea beetles and maggots exerted an important influence in the unscreened plats, killing many plants or so injuring them as to greatly retard their growth. Radishes pulled after June 1 were so maggoty that they were unfit to market.

The yields and weights of radishes in the screened and unscreened plats are given in Table III.

EXPERIMENTS DURING 1916.

During this year radish seed was sowed on the following dates: Plat 1, April 4; Plat 2, April 11; Plat 3, April 18; Plat 4, April 25; and Plat 5, May 6. After a cold, wet period, followed by very hot weather, flea beetles appeared in great numbers about May 20 and were very injurious to radishes, especially in the unscreened frames until the roots were harvested. On May 24 eggs of the radish maggot flies were noticed about unprotected plants in goodly numbers and, commencing with June 1, radishes became increasingly infested with maggots as the season advanced. The yields of the different plats are given in Table IV.

EXPERIMENTS DURING 1917.

For 1917 nine sowings of radish seed were made as follows: Plat I, Mar. 23; Plat II, Mar. 30; Plat III, April 6; Plat IV, April 12; Plat V, April 19; Plat VI, April 27; Plat VII, May 4; Plat VIII, May 10; and Plat IX, May 18. On May 9 feeding by flea beetles was first noticed, and on May 15 seedlings showed plainly evidences of injury, although the insects were not nearly as numerous or as destructive as in preceding years. Likewise maggots were not very abundant and, while the first adult of this species was observed on May 9, eggs and larvæ were not detected until the first week of June. Low temperatures and rains prevailed during the growing period of the radishes so that the season was very backward, at least

TABLE IV.— YIELD OF SCREENED AND OPEN RADISH BEDS DURING 1916.

(Counts and weighings based on rows of 100 feet in length.)

SCREENED.										UNSCREENED.									
Date of sowing.	Date of pulling.	Plants.	Total weight.		Market-able roots.	Weight of market-able roots.		Weight of leaves.		Date of sowing	Date of pulling.	Plants.	Total weight.		Market-able roots.	Weight of market-able roots.		Weight of leaves.	
		No.	*Lbs.*	*ozs.*	*No.*	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*			*No.*	*Lbs.*	*ozs.*	*No.*	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*
April 4	May 27	768	10	0	572	6	1	3	2	April 4	*May 27	725	5	3	361	2	8	1	5
											June 2	784	7	5	445	3	13	1	9
April 11	May 29	852	9	9	561	5	0	3	1	April 11	*May 29	896	6	1	376	2	12	1	5
											June 2	828	5	13	307	2	12	1	7
April 18	June 2	917	10	12	604	6	7	2	8	April 18	June 2	885	3	8	120	1	1	0	8
April 25	June 5	652	7	5	481	5	8	3	13	April 25	June 5	685	1	13	65	0	8	0	5
May 6	June 15	493	6	11	340	4	0	2	0	May 6	June 15	420	3	1	141	1	12	1	5
May 6	June 15	696	9	4	449	5	0	2	1	May 6	June 15	423	3	4	102	1	10	0	13
May 6	June 15	748	9	5	492	5	4	2	4	May 6	June 15	447	3	5	81	1	0	0	10
May 6	June 15	812	9	8	476	5	5	2	0	May 6	June 15	416	3	5	112	1	7	0	14

SUMMARY.

	Plants.	Total weight.	Roots marketable.	Weight of marketable roots.
	No.	*Lbs.*	*No.*	*Lbs.*
Average per 100 feet screened	742	9	496	5.3
Average per 100 feet unscreened	650	4.2	211	1.92

* At this date plants in unscreened beds had not grown as fast as the screened beds; so a later pulling was made in order that the comparisons should be fair.

two weeks behind the average. The temperatures in screened and open beds and conditions of growth of the plants are noted in Table V, while the yields of the different plats are recorded in Table VI.

TABLE V.— RECORD OF TEMPERATURES DURING EXPERIMENTS OF 1917 WITH NOTES ON GROWTH OF PLANTS.

DATE.	SCREENED PLATS.		UNSCREENED PLATS.		Remarks.
	Min.	Max.	Min.	Max.	
	Degs. F.	*Degs. F.*	*Degs. F.*	*Degs. F.*	
March 26......	26	62	28	60	Plat I was sown on March 23.
28......	27	64	26	66	
29......	31	41	32	41	
30......	26	44	28	45	Plat II was sown on March 30.
31......	28	49	28	49	
April 1......	.....	.....	.....	.....	
2......	37	67	38	66	A few seeds sprouting in both plats but no seedlings have pushed thru ground.
3......	30	61	30	61	
4......	30	55	30	50	No plants showing above ground.
5......	38	68	36	59	
6......	32	43	32	43	Plat III planted April 6.
7......	29	36	30	37	Screens covered with snow.
8......	.....	.....	.....	.....	
9......	20	44	22	41	Ground frozen solid, remaining so until April 11.
10......	22	42	23	41	
11......	25	43	27	42	No plants showing above ground in any of the plats.
12......	39	65	40	58	In Plat I plants beginning to push thru ground both outside and beneath screening. Rows not yet distinctly marked; little difference between screened and open beds. Plat IV sown on April 12. An occasional plant showing thru ground under screening, none outside.
13......	27	54	29	51	
14......	27	38	29	38	
15......	30	44	30	46	
16......	28	45	29	43	
17......	26	45	26	46	In Plat I outside sowing not up and entire plat not as far along as Plat II. In Plat II many plants under screening up, but rows not clearly indicated. In uncovered areas plants just beginning to push thru ground.

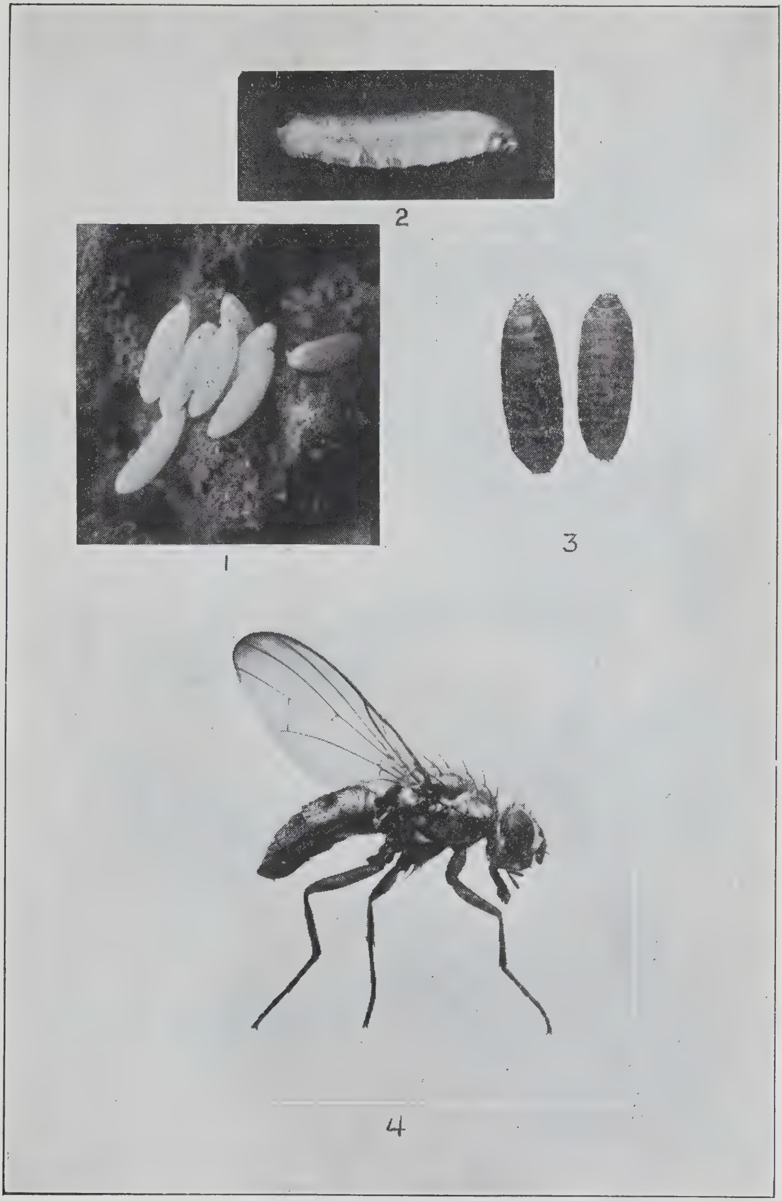

Plate XXXI.— Life Stages of Radish Maggott:
1, Eggs; 2, larva; 3, puparia; 4, adult female.

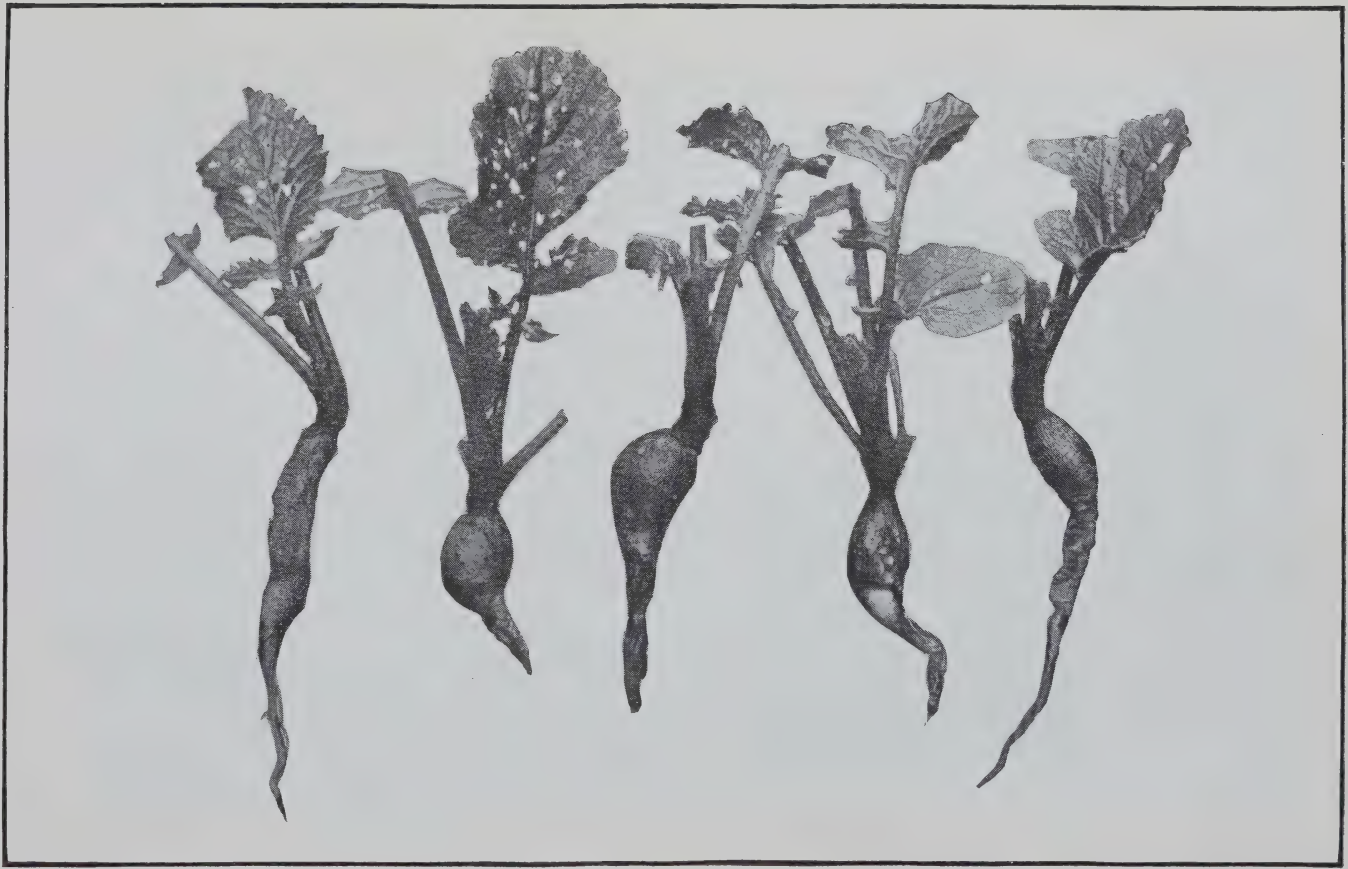

Plate XXXII.— Injurious Work of Maggot on Radish Roots.

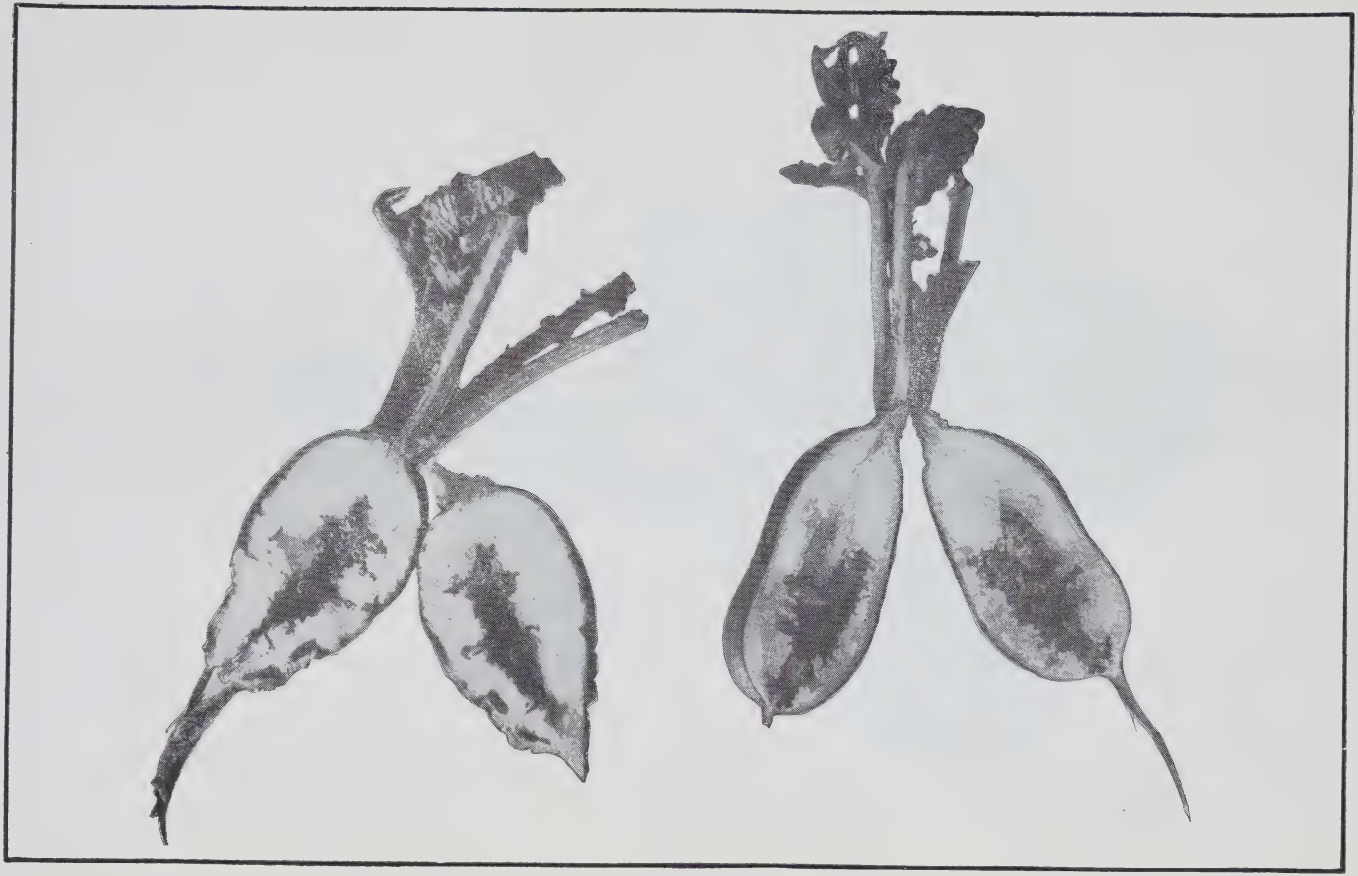

Plate XXXIII.— Maggot Injury in Interior of Radish Roots.

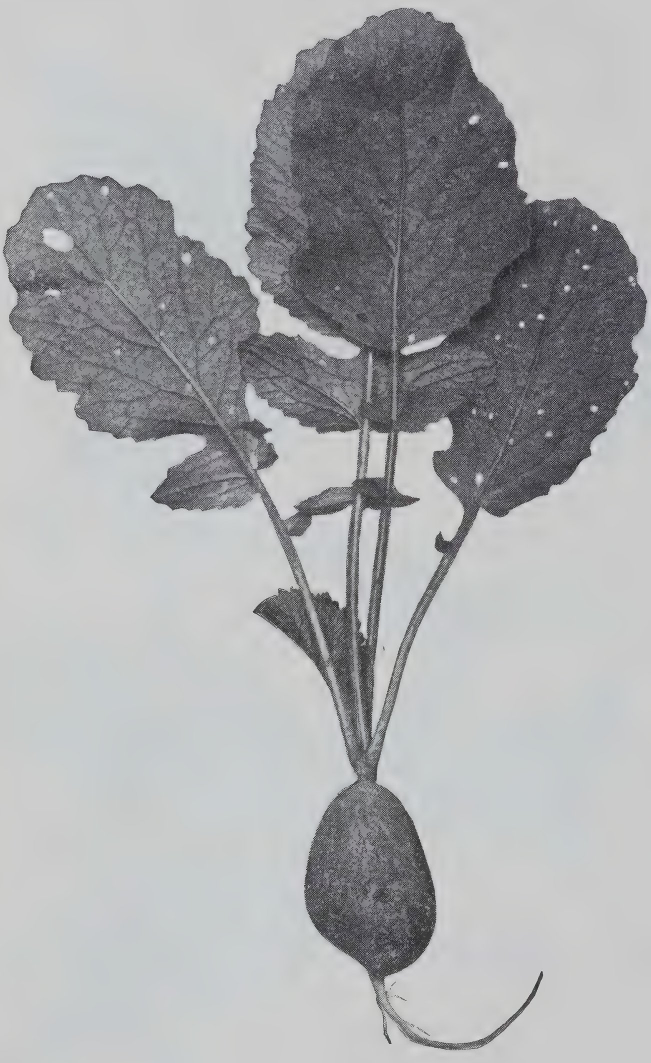

Plate XXXIV.—Radish from Screened Bed Showing Growth and Flea-Beetle Injury.

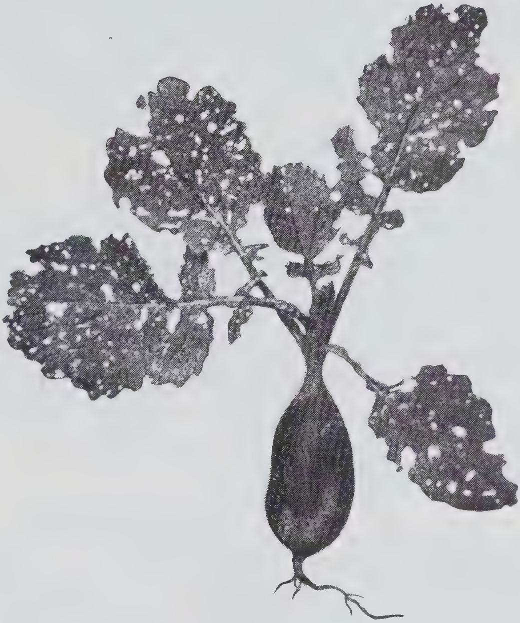

PLATE XXXV.—RADISH FROM UNSCREENED BED SHOWING GROWTH AND FLEA-BEETLE INJURY.

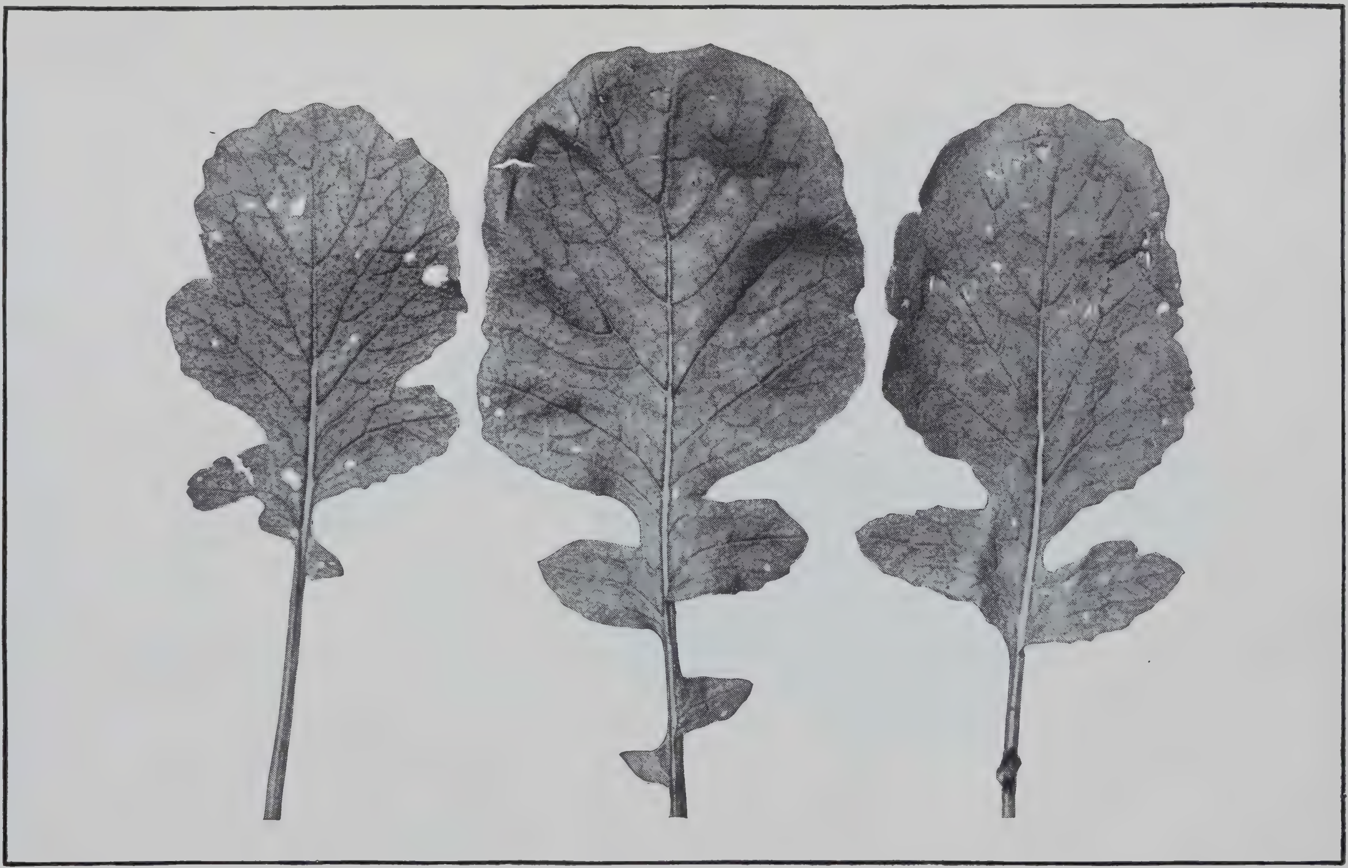

Plate XXXVI.— Typical Radish Leaves from Screened Bed.

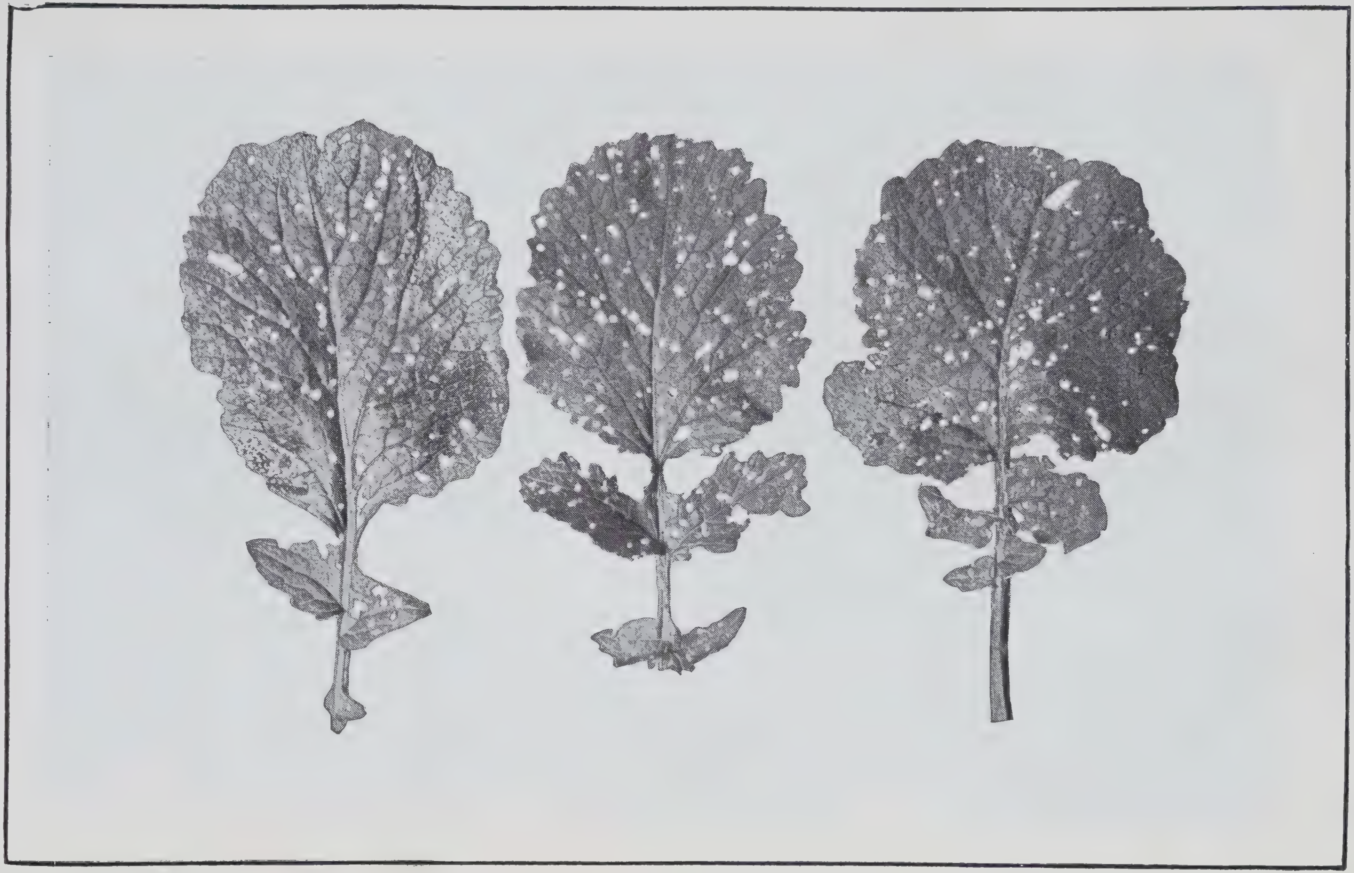

Plate XXXVII.— Typical Radish Leaves from Unprotected Plants.

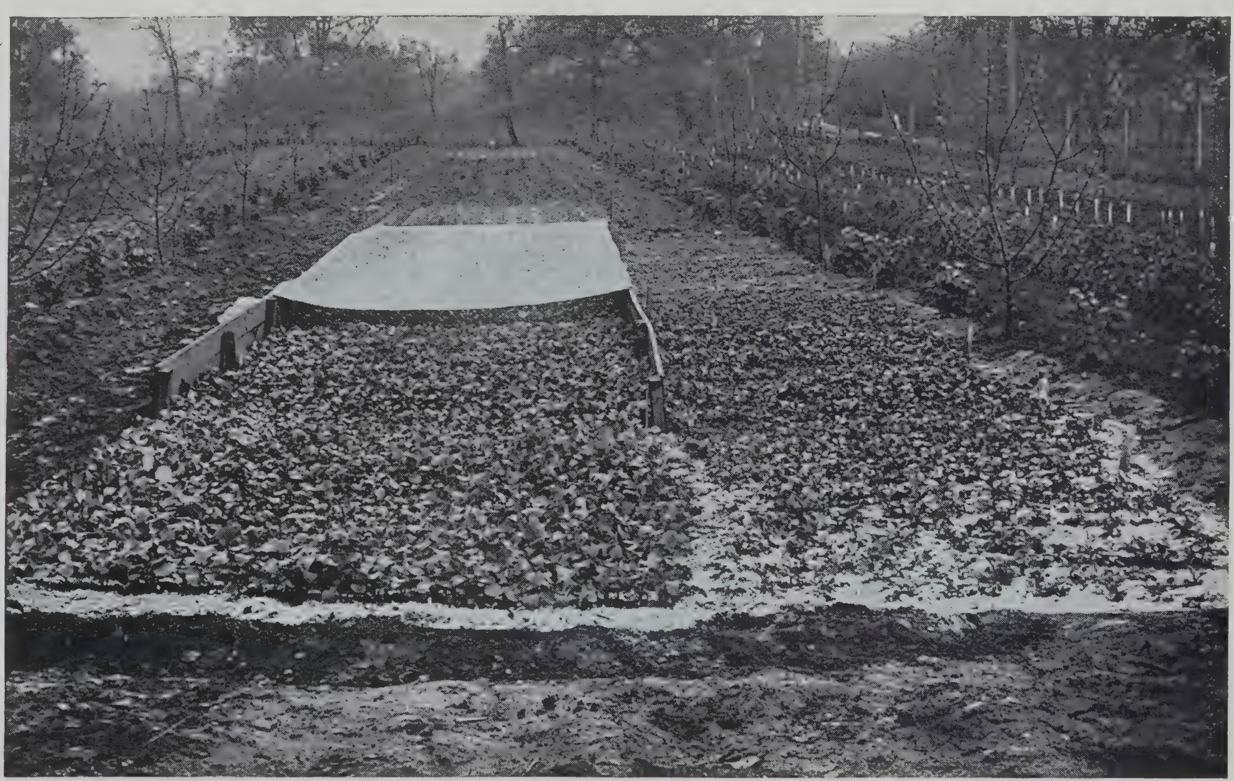

Plate XXXVIII.— Condition of Radishes in Screened and Unscreened Beds.

TABLE V.— RECORD OF TEMPERATURES DURING EXPERIMENTS OF 1917 WITH NOTES ON GROWTH OF PLANTS (*continued*).

DATE.	SCREENED PLATS.		UNSCREENED PLATS.		Remarks.
	Min.	Max.	Min.	Max.	
	Degs. F.	*Degs. F.*	*Degs. F.*	*Degs. F.*	
April 18......	35	74	34	63	In Plat I poor stand under screening, not as good as screened plants in Plat II; no plants showing in uncovered areas. In Plat II majority of plants up in screened frame and rows show distinctly, while in open bed only a few seedlings have appeared.
19......	47	79	47	71	In Plat I very poor stand under screening, while in open bed only an occasional plant shows. In Plat II an almost perfect stand in screened frame, while in uncovered bed about 20 per ct. of seedlings are up. Plat V sown on April 19.
20......	50	76	51	71	In Plat III, under screening, about 25 per ct. of plants are showing above ground while none have appeared in the open bed. In Plat IV an occasional plant shows in screened frame, but no seedlings are showing in uncovered bed.
21......	52	71	53	71	
22......	39	76	39	74	
23......	37	71	36	69	In Plat III plants well up, both inside and out. In Plat IV, plants practically as far along as in Plat III. Seedlings well up outside and beneath screening.
24......	31	63	31	57	
25......	29	65	25	54	In Plat V plants are coming up rapidly under screening; no plants as yet in open bed.
26......	41	64	42	62	In Plat V rows under screening well up. In open bed a few plants pushing thru ground all over bed.
27......	35	65	36	64	Plat VI sown on April 27. In Plat V about 30 per ct. of sowing is up in open bed.
28......	33	48	34	48	
29......	29	77	30	70	
30......	31	74	31	68	
May 1......	44	57	45	56	

TABLE V.— RECORD OF TEMPERATURES DURING EXPERIMENTS OF 1917 WITH NOTES ON GROWTH OF PLANTS (*continued*).

DATE.	SCREENED PLATS.		UNSCREENED PLATS.		Remarks.
	Min.	Max.	Min.	Max.	
	Degs. F.	*Degs. F.*	*Degs. F.*	*Degs. F.*	
May 2	38	55	38	54	
3	34	42	35	43	
4	32	50	32	50	Plat VII sown on May 4.
5	37	51	37	50	
6	38	52	38	51	
7	34	51	34	50	Seedlings in Plat VI just beginning to appear beneath screening. No plants showing in open bed.
8	39	62	39	58	In Plat VI rows under screening are distinctly marked. In open bed plants have not yet started.
9	40	69	41	62	In Plat VI seedlings in open bed just beginning to appear. Flea beetle injury first noticed, and first record of appearance of radish maggot fly.
10	36	66	37	63	Plat VIII sown on May 10.
11	35	62	35	61	
12	39	55	38	54	In Plat VII, plants beginning to appear under screening. In open beds no plants are showing.
13	41	59	41	56	
14	39	66	40	63	
15	35	71	36	68	In Plat VII, under screening, rows now distinctly marked. In open area plants beginning to push thru. Flea-beetle injury common, but few insects observed.
16	42	62	42	62	
17	36	56	36	54	In Plat VII, under screenings, rows all filled, plants well up. In uncovered area rows not yet clearly outlined. In Plat VIII an occasional plant up. No plants showing in open beds.
18	38	73	36	64	
19	39	78	40	76	In Plat VIII rows well up in screened area, but only a few plants showing in open bed.
20	53	85	54	82	
21	39	63	39	62	In Plat VIII rows almost all up in open bed. In Plat IX no plants up either under screening or outside. In Plats I and II some roots nearly of marketable size.

TABLE V.— RECORD OF TEMPERATURES DURING EXPERIMENTS OF 1917 WITH NOTES ON GROWTH OF PLANTS (*concluded*).

Date.	Screened Plats.		Unscreened Plats.		Remarks.
	Min.	Max.	Min.	Max.	
	Degs. F.	*Degs. F.*	*Degs. F.*	*Degs. F.*	
May 22......	43	68	44	63	
23......	45	57	46	56	In Plat IX, under screening, plants beginning to show; none in open area. In Plat VIII plants in open well up.
24......	37	55	37	56	
25......	36	50	37	49	In Plat IX, in uncovered bed, plants just pushing thru ground. Under screening rows are fairly well outlined.
26......	40	63	42	60	
27......	44	75	44	72	
28......	44	74	44	73	Pulled Plats II and III under screening.
29......	47	53	47	52	
30......	36	62	35	60	
31......	40	79	37	72	Pulled Plats I, IV and V under screening, and Plats I, II, III and IV in open beds.
June 1......	54	76	55	76	
2......	51	76	52	76	
3......	62	74	60	73	
4......	46	70	45	68	Pulled Plat V in open bed.
5......	51	84	52	80	
6......	51	67	51	68	
7......	50	76	49	75	
8......	50	75	48	74	
9......	50	69	49	71	
10......	59	80	58	79	
11......	53	74	52	74	
12......	50	69	48	67	Pulled both screened and open bed of Plat VI on June 12; Plats VII and VIII on June 15; and Plat IX on June 22.
Total temperatures........	2,914	4,724	2,927	4,558	
Average per day.	38.34	62.16	38.51	59.97	
Average daily difference....	Min. 0.17		Max. 2.13		

TABLE VI.— SHOWING YIELDS OF SCREENED AND OPEN RADISH BEDS DURING 1917.

(Counts and weighings based on rows of 100 feet in length.)

SCREENED PLATS.

No. OF PLAT.	Plants.	Weight of plants.		Weight of roots.		Marketable roots.	Weight of marketable roots.		Total weight of marketable plants.	
	No.	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*	*No.*	*Lbs.*	*ozs.*	*Lbs.*	*ozs.*
1	866	8	2	4	5	356	3	6	5	10
2	756	15	12	7	4	522	6	8	10	14
3	978	17	12	6	10	626	5	14	11	6
4	1,232	15	4	8	8	688	7	2	11	0
5	1,104	11	12	7	2	614	5	4	8	8
6	1,154	19	6	9	2	784	8	0	15	2
7	1,216	22	4	11	0	724	10	0	17	10
8	1,502	19	2	13	4	586	6	4	11	2
9	996	17	14	9	10	464	6	2	11	2
UNSCREENED PLATS.										
1	117	0	7	0	2	11	0	⅘		1½
2	651	14	0	8	13	385	4	5	6	8
3	1,199	10	4	6	5	517	5	3	7	8
4	1,093	13	14	8	0	570	6	0	9	5
5	1,161	12	8	7	13	853	7	5	10	6
6	1,490	19	12	11	2	924	10	0	16	0
7	1,048	12	2	6	10	562	5	14	9	2
8	1,336	14	0	9	8	588	5	2	8	0
9	1,471	21	0	10	13	705	8	5	13	10

SUMMARY.

	Plants.	Total weight.	Roots marketable.	Weight of marketable roots.
	No.	*Lbs.*	*No.*	*Lbs.*
Average per 100 feet screened	1,089	16.4	596	6.5
Average per 100 feet unscreened	1,063	13.1	568	5.8

GENERAL CONSIDERATIONS AND SUMMARY.

TIME OF OCCURRENCE OF INJURY.

The activities of the radish maggot in the different plats of the experiments, as described, are indicated in Table VII.

TABLE VII.— CONDITIONS OF PLATS WITH REFERENCE TO MAGGOT INJURY FROM 1914 TO 1917.

DATE OF SOWING.	Date of pulling.	Period of growth.	Conditions of plants.
1914, May 9	June 5	27 days	Badly infested.
1915, Mar. 15	May 19	65 days	No injury.
Mar. 22	May 19	58 days	No injury.
April 1	May 24	53 days	None to slight injury.
April 5	May 24	49 days	None to slight injury.
April 13	May 24	41 days	None to slight injury.
April 14	May 29	45 days	Considerable injury.
1916, April 4	May 27	53 days	No injury.
April 11	May 27	46 days	No injury.
April 18	June 2	45 days	Slight injury.
April 25	June 21	57 days	Badly infested.
May 6	June 21	46 days	Badly infested.
1917, Mar. 23	May 31	69 days	No injury.
Mar. 30	May 31	62 days	No injury.
April 6	May 31	55 days	No injury.
April 12	May 31	49 days	No injury.
April 19	May 31	†42 days	No injury.
April 19	June 4	‡46 days	Evidences of injury.
April 27	June 12	46 days	Slight injury.
May 4	June 15	42 days	Slight injury.
May 10	June 15	36 days	Slight injury.
May 18	June 22	35 days	Slight injury.

† Screened plat.
‡ Unscreened plat.

As will be observed in the foregoing table, the radish maggot has varied greatly in importance during the four years mentioned, causing considerable damage to the late plantings during 1914, 1915 and 1916, while in 1917 the extent of injury to roots was relatively insignificant. The injury was caused by the first brood of maggots as previously demonstrated by Schoene* in his study of the insect in cabbage plantings, and in the main chief losses of radish roots occurred during the latter part of May and during early June. Plantings that were ready to be pulled by the beginning of the last week in May escaped with little or no injury.

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* Schoene, W. J., N. Y. Agr. Exp. Sta., Bul. 419, p. 125. 1916.

EFFECT OF SCREENING ON GROWTH AND QUALITY OF ROOTS.

With the exception of 1917 when cold wet weather prevailed during the growing period most of the sowings in the screened beds showed a higher rate of germination of radish seed, which was largely due to the better physical condition of the soil. In the uncovered plats the surface soil was frequently dry and hard so that germination of seed was retarded, if not entirely checked, and young seedlings often failed to establish themselves. As a rule the seedlings in the open beds were one or more days behind the screened plants in the time of their appearance above ground. (See Table V.) Besides a larger and more even stand on the average the screened radishes also grew more rapidly and presented throughout the growing season a more vigorous appearance.

The period of growth of radishes in the covered frames from time of sowing to the harvesting of the roots was variable, being largely governed by the date of planting and seasonal conditions. An examination of Table VII shows that the time required for the production of marketable roots was as follows:

For sowings during March, 58 to 69 days.
For sowings during April, 41 to 57 days.
For sowings during May, 27 to 46 days.

The total average yields of the screened and open plats are tabulated in Table VIII.

Besides larger yields the roots grown in the screened frames were for the most part more succulent and tender and therefore a more satisfactory product for the market. In addition to smaller yields of roots the leaves of the unscreened plants have usually been small, leathery and prickly to the touch. The explanation for these differences is that, with the meteorological conditions that normally prevail during the months covered by these tests, screening modified the physical environment of the plants, principally as regards temperature and moisture conditions of the soil, which, as a rule, proved very congenial to them. On clay soils, such as used in these experiments, incrustation of the surface of the soil, accompanied by hot weather and slight precipitation, have proven, as shown by the behavior of the check plats, severe handicaps to the production of roots of good size and desirable quality.

Table VIII.— Summarized Yields of Screened and Unscreened Radish Plats from 1914 to 1917.

(Counts and weighings are based on rows of one hundred feet in length.)

		Plants.	Weight of plants.		Market-able roots.	Weight of market-able roots.	
		No.	Lbs.	ozs.	No.	Lbs.	ozs.
Averages for screened plats,	1914..	456	8	8	236	3	6
(Plat VI)	1915..	519	5	13	213	2	13
	1916..	742	9	0	497	5	5
	1917..	1,089	16	6	596	6	8
Averages for unscreened plats,	1914..	370	3	9	76	0	10
(Plat VI)	1915..	624	3	8	110	0	13
	1916..	650	4	3	211	1	15
	1917..	1,063	13	2	568	5	13
Total average of screened plats per 100 feet		702	9	14	386	4	8
Total average of unscreened plats per 100 feet		677	6	2	241	2	5

EFFECT OF SCREENING ON FLEA BEETLE AND MAGGOT.

The differences between screened and unscreened beds with respect to attacks by flea-beetles and maggots have usually been very marked. With the exception of 1917 each year that the tests have been conducted both pests have been sufficiently abundant to cause appreciable damage. The flea-beetles were usually first to appear and they attacked the cotyledons of the radish seedlings, eating small round holes which resulted in damage of varying intensity. Young seedlings frequently succumbed to the first onslaughts of the beetles and many other plants were so stunted by the repeated attacks of the insects that they never produced roots of marketable size. Another form of injury that escapes the attention of the average grower is the work of the larvæ on the roots, which is unquestionably of sufficient importance during some seasons to exert a retarding influence on the growth of the plants. While screening has not completely protected radishes from flea beetles there has been, on the whole, considerably less evidence of the work of these insects in screened beds than in the open beds; and in all attacks by the creatures in screened beds the amount of damage by them has been considered negligible.

With respects to maggots the contrasts between screened and unscreened beds have been uniformly more conspicuous than those produced by the flea beetles. By means of tight frames and proper attachment of cloth injuries by maggots have been completely avoided. As shown by reports of the experiments during the different years, maggots began to appear during the latter part of May and radishes in open beds maturing during early June have frequently been too much infested with maggots to be marketable. Besides rendering roots less desirable for purposes of consumption there have also been losses due to actual destruction of seedlings by maggots and retardation of growth and dwarfing of plants.

INFLUENCE OF SCREENING ON OTHER PESTS.

As grown under screening radishes possess tender foliage and roots. The plants are thickly massed, and the large leaves cause considerable shading of both plants and the ground about them. Cheesecloth restricts air circulation and reduces the intensity of light. There is, as a rule, more moisture in the soil, as probably of the air also, and temperatures range higher than in open beds. As compared with the latter method of growing radishes, it would appear that conditions under screening are more favorable for the development and growth of damping-off and downy mildew (*Peronospora parasitica* Pers.). While the latter disease has in occasional years, especially during 1917, caused quite a little damage to cabbage seedlings in screened frames, no noticeable injury has so far been observed with radishes.

Of more importance to date than the foregoing diseases are certain species of slugs and millipedes which, during seasons when there are prolonged spells of cold, wet weather, have proven capable of causing quite a little damage.

Three species of slugs have been collected about radish beds which, through the courtesy of Dr. F. C. Baker of the N. Y. College of Forestry, were identified as *Agriolimax agrestis* Linné, *Agriolimax campestris* Binney and *Limax maximus* Linné. All are known to obtain subsistence from various plants, including vegetables, but *A.*

agrestis has always been more abundant and has been largely if not entirely responsible for injuries to radish roots.

The species *A. agrestis* is of foreign origin, and in England where it occurs generally in gardens and fields it is known as the Field Gray Slug. It is one of the most prolific of its race. In 1905[1] it was stated that for four years this and other species had greatly increased all over Britain " so much so that whole fields of wheat and other plants had been destroyed. In gardens they had been even worse, and it has frequently been impossible to grow a crop of early peas or beans. Young potato sprouts and flowering plants of all kinds have suffered in a similar way."

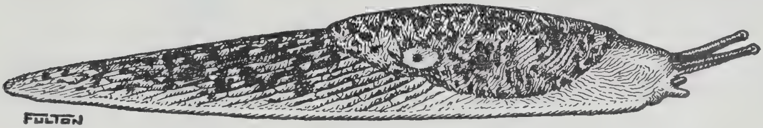

Fig. 11.—The Field Gray Slug (*A. agrestis*)

In the radish beds the slug *agrestis* fed upon both leaves and roots. In the attacks on foliage the injury has been variable, ranging from production of small holes through the leaf tissues to the entire destruction of leaves with exception of the stalks and larger veins. In the attacks on the roots the slug causes mere abrasions which appear as whitish specks on the surface of the radish and produces also holes of varying depth and circumference, ranging from one-fourth to one-half inch across. Owing to the white flesh which is exposed by the attacks of the creatures the eaten areas contrast strongly with the reddish epidermis. The slugs are nocturnal, and during the day they may be seen in protected situations about the frames or under stones or clods of earth or in the soil. They are partial to moisture, apparently preferring cool, damp situations. During cloudy weather they may be observed in exposed situations, even during the daytime. The chief damage to radishes has usually occurred during the latter part of May or during early June, or as fleshy roots were attaining marketable size. Until 1916 injuries to radishes had never been very conspicuous, but during the past two years the damage by slugs has been important. It should also be noted that in the region about Geneva *agrestis* has been quite common in gardens, being especially destructive to beans, lima beans particularly, as they are sprouting and becoming established, and to corn seedlings. Sprouts of such plants as dahlia and potato, shoots of salad plants like dandelion and chicory, and potato tubers have also been subject to attack. Damage to ripening strawberries has also been reported, which was probably by the same species. Aside from the work of the pest in gardens, farmers have complained of injuries to their field crops, particularly in plantings of corn and beans. It thus appears that the slug is an omnivorous feeder and is widely distributed. The conditions of its surroundings apparently largely determine its local abundance and importance.

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[1] Board of Agriculture and Fisheries, Leaflet No. 132.

While *agrestis* attacks radishes in both open and screened beds, it has been somewhat more destructive in the covered frames. From the well-known preference of slugs generally for shade and moisture, the conditions under screening would appear to have been more congenial in these respects for the activities of the creatures. In this connection it should be stated that in 1916 and 1917, when appreciable injury was done by them, rains and cold temperatures prevailed during the growing period of the radish, while in the two preceding seasons, when precipitation was not so great and temperatures were higher, they caused little harm. These experiences would indicate that the slug will vary in importance according to seasonable conditions, and that it will probably not prove an important pest each year. Efficient and practical methods of control have not so far been conclusively demonstrated. Feeding experiments with the slug to this end indicated that it prefers sliced potatoes to either radishes, strawberries, bran mash or clover bait. Sliced potatoes dusted with zinc arsenite and calcium arsenate were apparently eaten with relish, while potatoes treated with copper sulphate solution were distasteful. Tests with air-slaked lime showed conclusively that it is repellent to the slug. While no efforts have been made to subject the various measures to a severe test, it is

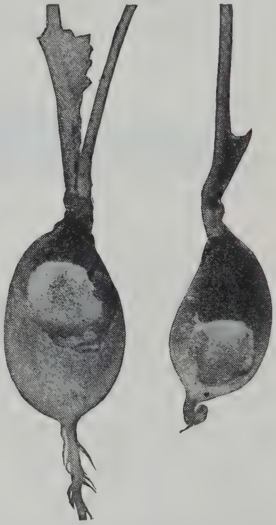
FIG. 12.— RADISHES INJURED BY SLUGS.

believed that collecting slugs by lantern light, applications of air-slaked lime in situations frequented by the pests, trapping with sliced cull potatoes, and spraying with bordeaux mixture, especially if an outbreak of mildew threatens, are measures that singly or in combination would afford adequate protection.

Of the millipedes, two species have been detected in radish beds, which are *Julus cœruleocinctus*[1] Wood and *Polydesmus moniliaris* Koch. The former has been more abundant and, as with the slugs, it has been most injurious during the past two years, which have been characterized by cold and wet weather during the growing season. Injury by *cœruleocinctus* is caused by its eating holes in the fleshy roots, which are similar in appearance to those produced by slugs. The damage has not been extensive, which is fortunate, as at the present time no practical measures have been developed for protecting radishes from attack.

CONCLUSIONS.

The practical outcome of the studies herein described is as follows: Radishes produced in the spring months in this latitude are subject to attack by one brood of maggots which in normal seasons injure roots during the latter part of May or early June. As this vegetable is grown in most home gardens these dates closely coincide with the period when radishes are making their most succulent root growth; hence the occurrence of maggots and attending injuries to the roots. By reason of this behavior of the insect two courses of action appear to be open to the grower who does not care to resort to applications of insecticides or to repressive measures — to dodge attacks by sowing either later than is customary or by sowing somewhat earlier than is the common practice. However, there is serious objection to the former step as radishes suffer from drought and heat incident to the summer and become fibrous and pungent. A recourse that is more to be preferred and which is quite practical is to practise early sowing, as timely planting of seed to secure harvesting of crop before the danger period has, in our experiments, generally resulted in the production of roots that were exempt from injury. In the adoption of this practise the caution is urged that in certain seasons, on account of weather and soil conditions, little leeway may be given as to choice of time for planting and it is not always possible to make as early sowings as are desirable to entirely safeguard the beds from attack. However, the gardener should make it a rule to plant radish seed as soon as the physical condition of the soil permits. For the protection of radishes that are to be harvested during the period of the prevalence of the insects, growing of radishes in frames covered with cheesecloth of 20 to 30 mesh has given excellent results. In addition to freedom from maggots, roots grown by this method have in most seasons given larger yields and have been more succulent and tender than those produced in open beds.

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[1] Identifications by Dr. R. V. Chamberlain, Museum of Comparative Zoology, Cambridge, Mass.

THE CHERRY LEAF-BEETLE.*

F. Z. HARTZELL.

SUMMARY.

The cherry leaf-beetle is a native species which normally feeds on the bird, pin, or fire, cherry (*Prunus pennsylvanica*). Both insect and plant have the same geographical distribution. Their habitat embraces Nova Scotia and Quebec westward thru southern Canada and the northern United States to the Rocky Mountains. It extends along the Alleghany Mountains to North Carolina and in the valley of the Fraser River to the Pacific Ocean. The beetle migrates for short distances beyond its normal breeding range.

The history of the species records intermittent attacks on cultivated cherries. The most extensive outbreak as regards extent of invaded territory and abundance of the insect occurred during 1915. The beetle was superabundant in two areas: (1) Western New York, Pennsylvania and northern West Virginia; and (2) the northern portion of the southern peninsula of Michigan. The outbreak in the Appalachian region extended as far east as Cayuga Lake in New York and Williamsport, Pa., and westward to the Ohio-Pennsylvania line, the infestation being most severe in the western portion of this area. In Michigan the beetle was most abundant in the region of Grand Traverse Bay. In 1916 and 1917 the numbers of the beetles in the foregoing areas were greatly reduced.

The eggs of this beetle are ellipsoidal in shape, averaging .78 mm. in length and .70 mm. in breadth, varying in color from stramineous to yellow. From these hatch olive-colored larvæ which later change to a very dark brown with the head and ninth segment black. When full grown the larva shows considerable yellow and characteristic black spots. The mature larvæ average about 7 mm. in length. The pupæ are bright yellow and vary from 4 to 5 mm. in length. The adults upon emergence are yellow, but soon change to yellow-brown and in a few days assume a blood-red color. They vary from 4.5 to 5.5 mm. in length.

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* Reprint of Bulletin No. 444, December, 1917.

The time of emergence from the pupal stage varies with seasonal conditions. During 1915 the adults emerged from August 23 to September 18, but during the summer of 1916, which was warmer, the adults appeared in the breeding cages from July 31 to September 2. The adults are rather sluggish, feeding very little during the late summer and fall. By September 15 some show a tendency to seek hibernating quarters, at least on cooler days, altho most of the beetles will emerge and feed on warm, sunny days. By October 1 all beetles entered hibernation, from which they did not emerge during the warm weather of early October.

The hibernation period of the insect in western New York is nearly eight months, emergence occurring during the latter part of May. In 1916, the first beetles emerged on May 27 at Fredonia. During 1917 at Lily Dale, eight miles from Fredonia and at an elevation five hundred feet greater, the first beetle emerged on May 30. The time of the appearance of the beetles was about one week after the bird cherry was in full bloom. The most extensive feeding by the adults occurs during the early part of June. It is at this time that practically all injury by the species to cultivated trees is inflicted. The greatest natural dissemination of the beetles occurs during the latter part of May and early June, when they may fly considerable distances to new feeding grounds.

Egg laying in 1916 began on June 5, under natural conditions, and on June 10 in observation cages, reaching the maximum in the first week of July and ending in the cages on August 9. The eggs are deposited on or near the trunk of the tree upon which the adults are feeding, usually not more than six inches above the surface of the soil, the majority being placed at the junction of the surface of the soil and the trunk. Some of the eggs are scattered loosely on the soil but most of them are glued to rootlets, small stones or the tree trunk. They are found to a depth of about one inch in the soil. The number of eggs laid in breeding cages by an individual varied from 10 to 294 with an average of 93. The normal life of the beetles appears to vary from 11 to 12 1-2 months, altho some individuals may reach an age of nearly 14 months.

The length of the incubation period during 1916 averaged 13 days, with a maximum of 23 days and a minimum of 9 days. These differences are ascribed largely to variation in temperature, altho there is individual variation in the incubation period of eggs deposited

on the same day. In 1916 hatching began on June 23 and ended August 20, the emerging larvæ being most numerous during the latter part of July.

Upon hatching, the larvæ climb trees and feed upon foliage. They are able to reach maturity only on the leaves of the bird cherry, and when compelled to subsist on the foliage of other species of cherry they invariably succumbed. The total feeding period of the larva varied from 8 to 24 days, with an average during 1916 of 12.3 days.

When the larvæ have reached full growth they burrow into the leaf mold or a short distance into the soil and form cells in which to pupate. The time spent in these cells was found to average 15 days, the shortest period being 12 days and the longest period 23 days. The total developmental period from hatching to emergence as adult averaged 27.2 days at Fredonia during 1916.

The chief factors in the natural control of the beetles are drowning of adults, reforestation which decreases the amount of the bird cherry, a carabid beetle (*Lebia ornata* Say) which attacks the beetles, and the cedar wax-wing (*Bombycilla cedrorum* Vieill) which was observed feeding on the adults. The cherry leaf-beetle is effectively controlled by arsenicals, preferably combined with bordeaux mixture, and nicotine sulphate; for the proper employment of which directions are given.

INTRODUCTION.

In view of the lack of detailed information on the bionomics of the insect, the occurrence of myriads of the cherry leaf-beetle (*Galerucella cavicollis* Le Conte) in fruit plantings thruout western New York in 1915 gave the initial impetus for a detailed study, which involved consideration of its origin, distribution, host plants, life history and susceptibility to control measures. This work has been conducted for three consecutive seasons, principally at Fredonia, which is located in the Lake Erie Valley with an altitude of five hundred to one thousand feet less than that of the Alleghany plateau where the species is normally of common occurrence. To insure accuracy the results of these studies were checked from time to time by observations of the beetle in its normal habitat. It should be stated that the extraordinary abundance of the insect led to simultaneous studies by other workers in this and several adjoining states, which will be duly noted elsewhere in this bulletin.

CLASSIFICATION AND SYNONYMY.

The cherry leaf-beetle belongs to an extensive family of beetles known as the Chrysomelidæ. It was described by LeConte who assigned it to the genus Galeruca. It was discussed in literature under the name *Galeruca cavicollis* until Horn, in 1893, made a critical study of the entire tribe and re-established the genus *Galerucella* which had been established by Crotch [1] in 1873 to include species closely related to *cavicollis* but which coleopterists did not recognize. Many of the species at present listed in this genus were originally described as members of the genus *Adimonia,* and some writers have referred to the cherry leaf-beetle under the appellation *Adimonia cavicollis.* The statements of Packard relative to *Galeruca sanguinea* were shown by Schwarz to refer to *cavicollis* [2] and Lugger's account of a cherry leaf-beetle under the name *Adimonia femoralis* obviously refers to this same insect.

The present synonymy of the insect is as follows:

Galerucella cavicollis Le Conte

Galeruca cavicollis. LeConte, J. L. Species of *Galeruca* and allied genera inhabiting North America (original description). *Proc. Acad. Nat. Sci. Phila.,* p. 216, 1865.

Galeruca sanguinea. Packard, A. S. Insects Injurious to Forest and Shade Trees, 5th Rept. U. S. Ent. Com., p. 529, 1890.

Galerucella cavicollis. Horn, G. H. The Galerucini of Boreal America. *Trans. Amer. Ent. Soc.,* **20**: 76-77, 1893.

Adimonia cavicollis. Davis, G. C. Rept. of the Consulting Entomologist. Mich. Agr. Expt. Sta., 7th Ann. Rept., p. 93, 1894.

Adimonia femoralis. Lugger, Otto. Minn. Agr. Expt. Station Bull. 66, pp. 236-238; also Ann. Rept. of Minn. Expt. Sta., pp. 236-238,1900.

HISTORICAL NOTES.

The cherry leaf-beetle was first described by Dr. LeConte [3] in 1865 from a single specimen collected in North Carolina. He named the insect *Galeruca cavicollis*, giving the description in Latin, but made no mention of its host plant. In 1893 Dr. Horn[1] redescribed *cavicollis* and placed it in the genus *Galerucella* where it has since remained. The known area of the distribution of the species is stated by Dr. Horn to be " from Canada to the New England and Middle States westward to Wisconsin; North Carolina (LeConte)." Zesch and Reinecke[2] in 1883 record the beetle under the caption *Galeruca cavicollis* as having been collected near Buffalo, N. Y.

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[1] Le Conte, J. L., and Horn, G. H. Classification of the Coleoptera of North America, pp. 348–349, 1883.

[2] Schwarz, E. A. *Insect Life,* 4:94. 1891.

[3] *Proc. Acad. Nat. Sci. Phila.,* 1865, p. 216.

The first mention of the habits of this insect is by Dr. Packard[3] who in 1890 recorded the insect as occurring in abundance at Berlin Falls, N. H., eating holes in leaves of " wild cherry." He also gave a brief description of the beetle, designating it *Galeruca sanguinea*, the name of a European species which it resembles. Schwarz[4] in 1891 called attention to the correct identity of the insect and said it is a common northern species.

The first recorded feeding of *cavicollis* on cultivated cherry is by Davis[5] in 1894 who observed the insect on this fruit at Bellaire, Mich. He designated the species *Adimonia cavicollis*, stating that a few beetles were observed feeding on wild cherry trees, and suggested the possibility of the species becoming a serious pest. He also described the larva for the first time. In 1896 Davis[6] again reported the appearance of the beetles in Michigan, which were in numbers sufficient to cause injury to cultivated trees. Mention is made of their feeding on the foliage of peach, cherry, apple and plum, and it is stated that arsenites were not satisfactory insecticides.

During the period of 1895 to 1899 inclusive the leaf-beetle seems to have been unusually abundant thruout the northern portion of its range and attracted the attention of a number of observers. However, Dr. John Hamilton[7] in 1895 reported the insect as rare in southwestern Pennsylvania but noted its occurrence on plants of the genus *Prunus*. In 1896 Dr. Lintner[8] reported having received specimens of this species from Ausable Forks, N. Y., on June 10,

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[1] *Trans. Amer. Ent. Soc.*, 20: 76–77. 1893.
[2] *Bul. Buffalo Soc. Nat. Sci.*, 4: 12. 1883.
[3] 5th Rept. U. S. Ent. Com. p. 529. 1890.
[4] *Insect Life*, 4: 94. 1891.
[5] Mich. Agr. Expt. Sta. 7th Ann. Rpt., p. 93; also *Insect Life*, 7: 200. 1894.
[6] Mich. Agr. Expt. Sta. 9th Ann. Rpt., p. 136. 1896.
[7] *Trans. Amer. Ent. Soc.*, 22: 371. 1895.
[8] 11th Rept. Ins. St. N. Y. pp. 197–198. 1896.

1895, from a correspondent who recorded the occurrence of "thousands" of the beetles on cultivated cherries and stated that "the beetles sent from cherry trees were also feeding on a young chestnut tree." The attacks of the insect on the latter host have never been verified. Lintner referred to the literature of the species and said, "the occurrence of this insect on the garden cherry while previously known only on the wild is another instance of the many similar changes known of our native insects from wild to cultivated food plants." Barrows and Pettit[1] in 1898 mentioned the insect as feeding on cultivated cherries in Michigan during 1897 and gave for the first time a general summary of its life cycle. For the prevention of injuries by the insect paris green, fish-oil soap and kerosene emulsion were advised.

In 1897 Johnson[2] stated that "myriads of the beetle and its larvæ were observed during the first week of September, devouring the leaves of the fire cherry (*Prunus pennsylvanica*) at Ricketts, Wyoming Co., Pa." Dr. Felt[3] in 1898 found the insect causing injury about Corning in Steuben County, N. Y. Dr. Smith[4] in 1898 observed the species feeding on peach in Pennsylvania and Chittenden[5] during the following year recorded injury to peaches at Lebanon and Spruce Creek in the same state. The account of the latter was the most comprehensive discussion of the species previous to 1915. In addition to the two localities just enumerated he recorded injury to cultivated cherry foliage at St. Ignace, Mich. He also gave a description of the egg and determined the incubation period for the latitude of Washington, D. C. Arsenicals were recommended for the protection of plantings. Dr Lugger[6] in 1899 reported the cherry leaf-beetle under the appellation *Adimonia femoralis* Melsh. as being numerous on "the native plum" and *P. pennsylvanica* in Minnesota, and Harvey[7] during 1900 recorded the insect by the name *Adimonia cavicollis* as being injurious to cherry in the vicinity of Orono, Maine, during 1899.

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[1] 37th Ann. Rept. Mich. State Bd. Agr. 1897–8, pp. 93–4. 1898.

[2] 3rd Ann. Rpt. Penna. Agr. Dept., Pt. II, pp. 106–7. 1897.

[3] *Country Gentleman*, 63:471; 14th Rpt. State Ent. N. Y. p. 235; U. S. Dept. Agr. Div. Ent. Bul. 17, n. s., p. 20. 1898.

[4] U. S. Dept. Agr. Div. Ent. Bul. 17, n. s., p. 23. 1898.

[5] U. S. Dept. Agr. Div. Ent. Bul. 19, n. s., pp. 90–93. 1899.

[6] Minn. Agr. Expt. Sta. Bul. 66, pp. 236–238. 1899. Minn. Agr. Expt. Sta. 5th Ann. Rept., pp. 152–154. 1899.

[7] Maine Agr. Expt. Sta. Bul. 60, p. 35. 1900.

For an interval of fifteen years, from 1900–1915, there is apparently no mention of *cavicollis* causing injuries in fruit plantings. However, during this interval publications were issued which deal either with groups of insects that attack certain plants or certain lists of Coleoptera, in which there are references to *cavicollis*. In 1901[1] and again in 1906[2] Felt mentioned feeding by the beetle on *P. pennsylvanica* in the Adirondacks. Pettit[3] in 1901 stated that the insect occurs thruout the upper peninsula of Michigan, and in 1905[4] he mentioned the species among insects injurious to the cherry. In 1902 MacGillivray and Houghton[5] reported *cavicollis* from Axton, N. Y., on the bird cherry. Washburn[6] in 1903 briefly mentioned the insect in a list of cherry pests. In 1909 Smith[7] listed the beetle as a pest on peach, plum and cherry. Blatchley[8] in 1910 gave tables for separating the several species of the genus *Galerucella* including *cavicollis* found in the state of Indiana. Mention was also made of its distribution, giving the range only east of the Mississippi River. In 1911 Gossard[9] gave remedies for the control of the pest, listing it with other cherry insects but made no mention of any outbreaks occurring in Ohio. In 1912 O'Kane[10] referred to the beetle as a destructive insect of cherry and gave a partial life history and remedies.

The outbreak during 1915, from the standpoint of numbers of beetles and the extent of territory infested is the most extraordinary on record for the species. On account of the attention that was directed to the insect by its superabundant numbers over a large area in several states, a number of contemporaneous and independent investigations were initiated.

Dr. Surface [11] recorded the insect as destructive to cherry, apple and other plants in Lycoming County, Pa., during 1915, and Parrott [12] gave an account of the species in New York, briefly summarizing its life history and experiments on control measures.

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[1] 17th Rept. State Ent. N. Y., p. 861.
[2] N. Y. State Mus. Memoir 8, vol. 2, p. 550.
[3] 40th Ann. Rept. State Bd. Agr. Mich., p. 192.
[4] 44th Ann. Rept. State Bd. Agr. Mich., p. 312.
[5] *Ent. News*, 13:252.
[6] Minn. Agr. Expt. Sta. Bul. 84, p. 96.
[7] Rept. N. J. State Mus., p. 347. 1909.
[8] Coleoptera of Indiana, p. 1169.
[9] Ohio Agr. Expt. Sta. Bul. 233, p. 129.
[10] Injurious Insects, p. 263.
[11] Monthly Bul. Div. Zool. Pa. Dept. Agr., vol. 5, pp. 20–21; vol. 6, p. 104.
[12] N. Y. Dept. Agr. Cir. 130, pp. 174–176; Proc. 61st Ann. Meeting West. N. Y. Hort. Soc., pp. 115, 123.

In 1916 Herrick[1] and Matheson described the egg, larval and pupal stages of the beetle with careful detail and outlined its life history. Crosby[2] also gave an epitome of the habits and life history with remedial measures. During the same year Hartzell and Parrott[3] published a circular giving a brief summary of the habits of the beetle, outlining methods of control based on experiences of the previous year. Dr. Felt[4] gave an account of the outbreak during 1915 in New York and a hypothesis for the sudden dispersion of the beetles. Also during 1916 Cushman and Isley[5] published the more important results of their studies to date on the habits and life history of *cavicollis,* especial attention being given to the larval and pupal stages, and on experiments for the control of the pest. Detailed descriptions were given of all the immature stages and extensive data on the duration of the larval instars and the pupal stage for large numbers of individuals. A bibliography was included by them in this study.

FOOD PLANTS.

In any discussion of the leaf beetle the distinction should clearly be made between the food plants upon which the larvæ are able to develop and those plants on which the adults occasionally feed. For convenience of reference the former are designated the *essential food plants* while the latter are denominated the *incidental food plants.*

There appears to be only one species of plant which is an essential food plant of *cavicollis* and this is the bird, pin, or fire, cherry, *Prunus pennsylvanica.* The larvæ of the leaf-beetle have never been observed to reach maturity on any other plant. This cherry is also the primary food-plant of the adult, and from present knowledge it is believed to be the only plant on which the beetle feeds during periods when conditions are normal. Most observers, as will be noted in the discussion dealing with the history of the beetle, record this species of cherry as the food plant of the adults. When the foliage of the bird cherry is destroyed or is of insufficient quantity the insects do feed upon other species of plants. Among cultivated trees the most important incidental food plants are the peach and sour cherry. All varieties of peach seem to be attacked altho the most serious damage results to young trees owing to the paucity of foliage as compared with the numbers of beetles on the leaves. Sour cherries, as Early Richmond and Montmorency, which are the principal varieties grown in western New York, exhibited considerable evidence of feeding by the insects, and of these two plants some observers claim that the former was preferred. The foliage of mature sweet cherries, according to our observations, was not attacked by the beetles, but several cases of partial defoliation of young sweet cherries were brought to our attention. Varieties of cherries intermediate between sweet and sour sorts, such as the Duke cherries, were quite attractive to the beetles. Observations during 1915 seem to indicate that the age and physiological condition of the tree are perhaps more potent factors than the variety of cherry in determining the preferences of the beetles. Young trees and older trees which were in a weakened condition were especially susceptible to their attacks. At Fredonia the beetles were observed eating the foliage of shoots that had grown from below the graft, which proved to be of the species *P. mazzard.* In the vicinity of Geneva the insect was quite abundant on *P. mahaleb*, apparently preferring it to cultivated trees. This cherry has escaped from cultivation and may be found growing along fences and in waste places. Mr. L. F. Strickland,[1] Inspector for the N. Y. Department of Agriculture, found the beetles attacking stocks of *P. mahaleb* in a nursery at Batavia. Davis [2] reported the beetles feeding on apple at Bellaire, Michigan. Recently Surface [3] gave a similar report of the adults in Lycoming County, Pa. In view of the foregoing statements it should be noted that frequent observations by the author failed to reveal any feeding of the beetles on apple foliage on which they were found during 1915. Cushman [4] and Isley have stated that "the apple was entirely immune" during the attack of 1915 as judged by conditions in Erie County, Pa. A number of observers claim that the beetle feeds on plums but there is reason to believe that other injury to the foliage has perhaps been mistaken for the work of this insect.

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[1] *Jour. Agr. Research,* 5: 943–949.
[2] Dept. Agr. State N. Y. Bul. 79, pp. 1148–1149.
[3] N. Y. Agr. Expt. Sta. Cir. 49.
[4] N. Y. State Mus. Bul. 186, pp. 83–84.
[5] U. S. Dept. Agr. Bul. 352.

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[1] Letter of June 27, 1916.

[2] *Insect Life*, 7:200. 1895.

[3] Monthly Bul. Div. Zool. Pa. Dept. Agr. 5:21. 1915.

[4] Loc. cit., p. 2.

Results of feeding on *Prunus serotina* were noticed by W. T. Davis[1] at Rock City, N. Y., during 1915. In the vicinity of Fredonia, N. Y., the beetles avoided the choke cherry, *P. virginiana*, and the wild black cherry, *P. serotina*, during 1915 and 1916. These species are the most common wild cherries in that region; and careful examination was repeatedly made for eggs and larvæ on them since a portion of the literature dealing with the insect reported it as occurring on " wild cherry " without any apparent distinction as to choice of species. Lugger reported this insect feeding on a " native plum " in Minnesota. Wight[2] records only two species of native plums in that state, *Prunus nigra* and *P. americana;* therefore it is probable that one of these two species is also an incidental food plant of *cavicollis.*

During the invasion of western New York the leaf-beetles were observed on potatoes, timothy, roses, dahlias, plums, grapes and milkweed (*Asclepias syriaca* and *A. purpurascens*). Careful observations failed to reveal any feeding on these plants. It, therefore, appears that the insects merely alighted on such plants to rest after a sustained flight and that later they sought the foliage of cherries and peaches to feed. It is highly probable that the reports in literature of the beetles feeding on cultivated plums, buttercups and chestnut, as previously mentioned, were prompted by the presence of the insects on such plants rather than by any evidence of actual eating of the foliage.

ORIGIN AND DISTRIBUTION.

The cherry leaf-beetle is native to North America. Its breeding range comprises Nova Scotia, Quebec and the region westward, embracing southern Canada and the northern states to the Rocky Mountains. (Fig. 13.) The southern limit of the species extends from southern New Jersey thru eastern Maryland; thence southwest along the eastern portion of the Alleghany highlands thru Virginia and North Carolina where it turns westward and later northward following the western border of the highlands to Lake

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[1] Letter of April 3, 1916.

[2] Wight, W. F. Native American Species of Prunus, U. S. Dept. of Agr. Bul. 179. 1915.

Erie in the vicinity of Erie County, Pennsylvania. Across Lake Erie, the line follows the northern shore for a distance of nearly 150 miles, then starts on the southern shore of the lake in the vicinity of Sandusky, Ohio, and extends westward thru Ohio, Indiana, Illinois and Iowa, thence northwest to the Black Hills of South Dakota where it bends sharply to the south, extending along the eastern slopes of the Rocky Mountains thru Wyoming and into Colorado perhaps to near the southern portion of the state. The northern limit of the beetle extends southwestward thru the northern portion of the St. Lawrence Valley to the northern shores of Georgian Bay and Lake Huron, crossing the Sault St. Marie river and following the southern shore of Lake Superior to the vicinity of Keweenaw Point. Beginning on the northern shore of the lake near Port Arthur the line extends northwestward to the north of Lake Winnipeg; thence westward thru central Manitoba, Sas-

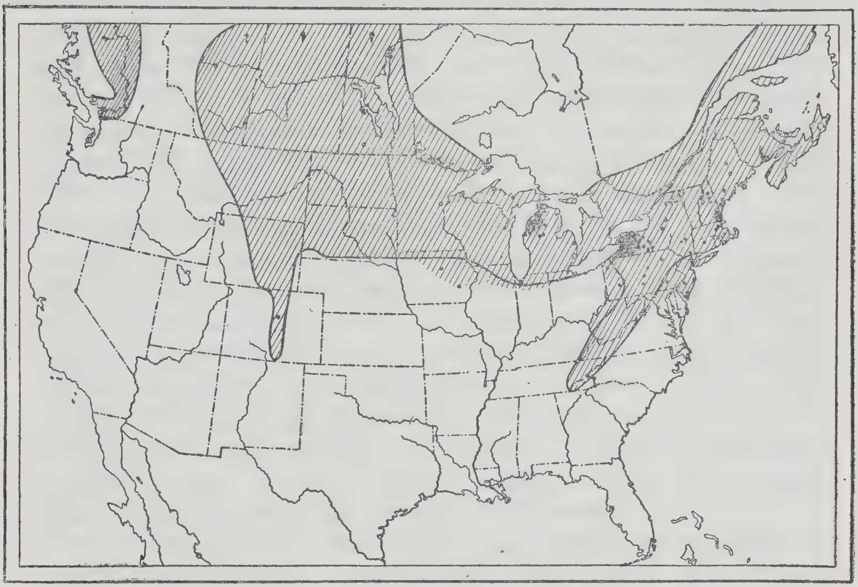

Fig. 13.— Map Showing the Distribution of the Cherry Leaf-beetle and Prunus Pennsylvanica.

The shaded area represents the probable range of *Galerucella cavicollis*, localities in which the beetles have been found shown by dots. The curved lines show the limits of the bird cherry and also define the breeding range of *G. cavicollis*. Distribution of the bird cherry east of the Rocky Mountains after Wight and Robbins, west of the Rockies adapted from Sargent.

katchewan and Alberta to the Canadian Rockies. The only record of the insect west of the Rocky Mountains is at Vancouver, B. C., but there is reason to believe that it may occur in other parts of British Columbia. In correspondence or in literature the beetle has been reported from the following localities:— Nova Scotia, Truro; New Brunswick, St. John; Ontario, Toronto; Manitoba, Aweme (Wickham); Alberta, Edmonton (Wolcott); British Columbia, Vancouver (Chittenden); Maine, Orono (Harvey), Mt. Desert (Fall), Ogonquit, Old Orchard, and Mt. Katahdin (Wickham), Monmouth (collected by Frost and reported by Wolcott); New Hampshire, Farmington, Haverhill and Exeter (Fall), Berlin Falls (Packard, Chittenden), Mt. Washington (Chittenden, Wickham), Hampton and Durham (Wickham), Mt. Adams (Chittenden); Vermont, (Leng); Masschusetts, Framingham (collected by Frost and reported by Wolcott), Cambridge and Mansfield (Chittenden); Rhode Island (collected by Davis, data by Wickham); Connecticut, Hartford (Chittenden); New York, localities not included in outbreak of 1915, Whiteface Mt. (W. T. Davis, Leng), Ausable Forks (Lintner), Axton (McGillivray and Houghton), Saranac, Old Forge and Newport (New York State Museum), Catskill (Chittenden, N. Y. S. M.), High Peak, Catskill Mts. and Peekskill (Leng), Buffalo (Zesch and Reinecke), Corning (Felt), Ithaca (Chittenden); Pennsylvania, Ricketts (Johnson), Lebanon, Spruce Creek, Allegany, (Chittenden), Williamsport (Surface, Cushman and Isley), Lycoming County (Surface), State College and Center County (Fagan), Northeast (Cushman and Isley), Dicksonburg (Backus); New Jersey, Atco, Anglesea, and Sea Isle (Smith); Virginia, Woodstock (Chittenden); North Carolina (Le Conte); West Virginia (Cushman and Isley); Ohio, Charles Drury [1] writes from Cincinnati as follows: "*Cavicollis* has not occurred here that I know of. In the many years that I have collected Coleoptera here I have never seen the species . . . . In collections from the central and northern part of Ohio which I have identified there were no specimens of *cavicollis* although the species should occur there." Cushman and Isley record the absence of the beetles in the northern part of the state as far west as Sandusky during 1915; Michigan, Bellaire (G. C. Davis and Pettit), Au Train Falls (Pettit), St. Ignace (Chittenden), Bay View (Liljeblad, data by Wolcott), Rudyard, Frederick, Empire,

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[1] Letter of April 13, 1916.

Isle Royal (Pettit), other records in account of outbreak of 1915; Indiana, Pine (Wolcott); " Probably to be found thru the northern third of state " (Blatchley); Wisconsin (Horn); Bayfield (Wickham); Iowa, Iowa City and Waterloo (Wickham); Minnesota, Hinckley (Lugger); Colorado, Palmer Lake (Gillette, Wickham); Texas, (Chittenden).

The southern limit of the range of this beetle in the Appalachian highlands is obscure. The type specimen is stated by LeConte to have been taken in North Carolina. There is no other record of the species from this area. Prof. Franklin Sherman Jr.[1] states that " the occurrence of *cavicollis* in this state rests on the statements of Le Conte and no specimens of the species exist in our collections." Regarding South Carolina, Prof. G. M. Amedi [2] writes, " we do not know that *cavicollis* occurs in this state." From the standpoint of definite records, Woodstock, Va., is the most southern point from which the beetle has been noted. Only one record of the occurrence of the leaf-beetle from the extreme northern limits of the normal habitat of *Prunus pennsylvanica* has come to our attention. This specimen was captured at Edmonton, Alberta, Canada, on July 6, 1912, and is now in the collection of Mr. A. B. Wolcott, Field Museum of Natural History, Chicago, who writes,[3] " This specimen is not typical, the occiput being piceous, but I believe it certainly is *G. cavicollis* as repeated study of the specimen has failed to lead to any better determination."

The breeding range of the beetle, from the standpoint of altitude, deserves consideration. In the east the insect has been found near the tops of our highest mountains. Prof. H. F. Wickham has specimens in his collection which were obtained from Mt. Katahdin, Me., at an altitude of 5,000 feet and from Mt. Washington, N. H., at altitudes ranging from 5,000 to 6,000 feet. The altitudes of these two mountains are respectively 5,273 and 6,293 feet. Data bearing on the highest altitude of the growth of bird cherry on these two mountains we have been unable to procure. Mr. Wm. T. Davis [4] writes, " in my visit on July, 1914, to Whiteface Mt., Adirondacks, which has an altitude of 4,872 feet, I found *cavicollis* in considerable

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[1] Letter of March 20, 1916.
[2] Letter of March 24, 1916.
[3] Letter of March 20, 1916.
[4] Letter of April 3, 1916.

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numbers at the top of the mountain as well as along the trail. It was destructive to the foliage of *Prunus pennsylvanica.*"

The highest elevation of the Adirondacks is Mt. Marcy which has an altitude of 5,344 feet. Dr. Bray[1] describes an Arctic flora existing on the summit of this mountain above 5,000 feet, and a few of the indicator species of shrubs which he gives are bearberry willow (*Salix uva-ursi*), glandular birch (*Betula glandulosa*), and Lapland rose-bay (*Rhododendron lapponicum*). Dwarf balsam fir and spruce forms a conspicuous portion of the vegetation on part of the summit, while a heath-mat type of vegetation, chiefly Lapland rose-bay, is dominant on another portion of the summit of this mountain. The bird, or fire, cherry was not found growing above 5,000 feet by this author.

It would appear from these records that the beetle thrives at an altitude as high as 5,000 feet in the northeastern United States. The altitude of the breeding range in the Rocky Mountains has not been determined. In Colorado *P. pennsylvanica* grows at elevations ranging from 4,000 to 9,500 feet.[2] The beetle has been collected near Palmer Lake, Colorado, at an elevation of 7,500 feet; and as the bird cherry grows at this elevation it would appear that this terrain is in the breeding range of the beetle in this state.

The records give the migratory distribution of the cherry leaf-beetle only south of the breeding range. There are no records which show that the beetles migrate north of the breeding range. The migratory range (Fig. 13) extends thruout the southeastern portion of Pennsylvania and southward in New Jersey as far as Anglesea; also in western Pennsylvania two localities — Allegheny and Dicksonburg — appear to occur outside the breeding range. Another apparent extension occurs in southeastern and central Iowa. This evidently is a migratory region because the bird cherry is not believed to be present except as isolated trees planted by man, and the beetle is rare in this locality. Prof. R. L. Webster,[3] Ames, Iowa, writes "*cavicollis* is not represented in the collection here. I do not believe that the insect has ever caused damage in Iowa."

Inasmuch as the beetles migrate such short distances from their breeding range, it is difficult to explain the finding of the beetles in

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[1] Bray, W. L. The Development of the Vegetation of New York State. Tech. Pub. No. 3, N. Y. College of Forestry, Syracuse University, pp. 77–79. 1915.

[2] P. A. Rydberg. Flora of Colorado. Col. Agr. Expt. Sta. Bul. 100, p. 193. 1906.

[3] Letter of March 21, 1916.

Texas, except on the hypothesis that there were undiscovered areas near this locality in which *P. pennsylvanica* grew at the time the beetles were taken. It is possible that in this locality the insect breeds on a closely related species of Prunus.

The correspondence between the range of the cherry leaf-beetle and the range of *Prunus pennsylvanica* is rather striking. The map (Fig. 13) illustrates the range of the bird cherry east of the Rocky Mountains as given by Wight [1] except the southern part of Colorado which data has been furnished thru the kindness of Prof. W. W. Robbins. Wight says, " *Prunus pennsylvanica* ranges from Newfoundland and New England westward through the valley of the St. Lawrence, Pennsylvania, northern Ohio and northern Indiana to the Black Hills and the eastern slopes of the Rocky Mountains, where it passes into the sub-species. It apparently does not occur north of Lake Huron, but extends northwestward from the western end of Lake Superior to the Winnipeg Valley. It is reported to extend to the shores of Hudson Bay (Sargent, Silva of North Am. 6:66), but no specimens have been seen from that region. It is common in the Alleghanies from Pennsylvania southward to southwestern North Carolina and is said to reach its greatest size in this region." Prof. W. W. Robbins [2] writes, " *Prunus pennsylvanica* in Colorado is found at altitudes from 4,000 to 9,500 feet along the whole eastern front of the mountains from the Wyoming line to the New Mexico line. I have never seen it west of the Rocky Mountain range. It does occur eastward on the plains but is found particularly on the rock outcrops, and in gulches." In addition to the foregoing distribution the bird cherry [3] is found on "the eastern slopes of the Coast Range of British Columbia in the valley of the Fraser River."

The larvæ of this insect are not known to develop on any plant except the bird cherry, so, provisionally, we have limited the breeding range of the beetle to the regions inhabited by this tree. The occurrence of *cavicollis* in those areas where *P. pennsylvanica* does not exist (Fig. 13) indicates that a distinction should perhaps be drawn between the breeding range and the migratory range of the insect.

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[1] Wight, W. F. Native American Species of *Prunus*, U. S. Dept. of Agr. Bul. 179, pp. 60–61. 1915.

[2] Letter of Nov. 7, 1917.

[3] Sargent, C. S. Silva of North America, 4:36, New York. 1893.

ACCOUNT OF RECENT OUTBREAK.

CONDITIONS IN WESTERN NEW YORK IN 1915.

During the first two weeks of June, 1915, the cherry leaf-beetle appeared in countless numbers on cherry and peach trees thruout western New York. In Chautauqua County the beetles were first noted in fruit plantings during the week ending June 5. At that time they were especially numerous in the southern part of the county where they swarmed on the foliage of many kinds of vegetation. Their presence was soon discovered by fruit growers who appealed for assistance to the various state institutions engaged in the promotion of agriculture. The exact date of the beginning of the invasion was not noted but by June 3 the beetles were sufficiently numerous to injure the foliage of cherry and peach trees at Jamestown. They were observed at Fredonia on June 7. At that date the beetles were present in myriads and continued to increase in abundance for nearly a week. Young sour cherry and peach trees were overrun with the insects and in a few days considerable injury was done to foliage. The most serious damage occurred in orchards nearest the localities in which the pin cherry was abundant. During the period of dispersion the beetles flew great distances and alighted on all manner of vegetation from which they later went to incidental food plants.

The beetles came from waste land and land denuded of forests which are grown over with *Prunus pennsylvanica* in the more mountainous portions of southern Chautauqua County and northern Pennsylvania; and the direction of their flight was northward. So prolonged and direct was their migration that vast numbers of the insects were carried far out into Lake Erie. Many alighted on drift-wood and other floating objects and eventually drifted ashore, where they sought food. Of those which fell in the water and were washed ashore, a small percentage revived but the majority perished. A visit to the shore of Lake Erie at Westfield, on June 23, revealed the dead bodies of millions of beetles in two parallel rows as far as the eye could see along the beach. On the drift-wood which lay on the beach the living beetles were so numerous that it presented a reddish appearance. Similar conditions were reported from the entire Chautauqua County shore of the Lake. On June 10 Cushman and Isley made similar observations on the shore of this lake in Erie County, Pennsylvania. As an example of popular misconception, it should also be noted that the presence of the beetles on the beaches and in the water gave rise to the idea that the insects came from Canada.

The dispersion was at its height in the vicinity of Fredonia from June 7 to 12. After the latter date the beetles were occupied with feeding and propagating. They fed ravenously until about June 15, when they became appreciably less active in this particular.

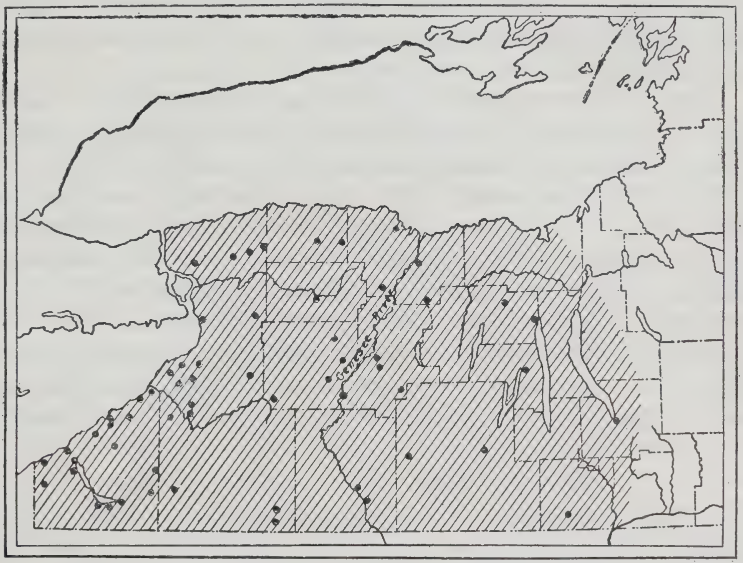

Fig. 14.— Map Showing the Extent of Infestation by the Cherry Leaf-beetle in Western New York During 1915.

Localities in which the beetles were observed or reported are shown by dots.

After June 20 little harm was done by the adults except on young trees. The beetles diminished in numbers during July and by the middle of August all had practically disappeared from cultivated trees.

While the leaf-beetle is indigenous to New York, destructive attacks on cultivated varieties of cherries have only been of local importance and at wide intervals and there is no record of as extensive an outbreak in this State as the one just described. The map (Fig. 14) illustrates what is believed to be the extent of the affected area in the State during 1915. It will be noted that the pest made its appearance over practically all of the western part of the State as far east as Ithaca. From observations and correspondence the insect appears to have been most numerous in the region west of the Genesee River and it was generally destructive thruout this area. The greatest number of inquiries from fruit growers came to our attention from Chautauqua and Erie counties. However, in the counties of Niagara, Orleans, Livingston and Monroe, where there are large acreages of peach orchards and nurseries many communications were sent direct to the State Horticultural Inspectors who reported that they were flooded with inquiries regarding the pest during the height of the invasion.

The insects were either observed by us or reported by correspondents to be present in conspicuous numbers at Randolph, Conewango Valley, Jamestown, Lakewood, Mayville, Ashville, Findley Lake, Ripley, Westfield, Brocton, Fredonia, Dunkirk, Cassadaga, Sheridan, Silver Creek, Irving, Angola, Brant, North Collins, Eden, Derby, Buffalo, Alden, Sanborn, Lockport, Gasport, Middleport, Albion, Holley, Batavia, Arcade, Wellsville, Dansville, Mt. Morris, Rochester, Hilton, Clifton, Clifton Springs, Geneva and Penn Yan. Wm. T. Davis[1] states that he took specimens at Rock City (Four Mile P. O.), Portage, and in Yates County during June, 1915. Herrick and Matheson[2] give the following additional places from which the species was reported as destructive during this period: Sonyea, Perry, Scio, Honeoye Falls, Bath, Holland, Collins, Gowanda, Wyoming, Perrysburg, Chautauqua, Kennedy, Castile, Elmira, Hornell, Olean and Ithaca. All places mentioned are indicated by means of dots on the map shown in Fig. 14.

OCCURRENCE OF LEAF-BEETLE IN OTHER STATES.

There were two separate regions in which outbreaks of *cavicollis* occurred during 1915; one comprising the Alleghany highlands of New York, Pennsylvania and northern West Virginia and the other the Grand Traverse Region of Michigan.

The beetles were especially destructive in the northern counties of Pennsylvania adjoining Chautauqua, Cattaraugus and Allegany Counties of New York. Surface[3] records the beetles as destructive in Lycoming County, Pa., which indicates that the outbreak extended as far east in the latter state as in New York. The injury by the beetles in Erie[1] County, Pa., resembled that in Chautauqua County. The outbreak extended thru the highlands of Pennsylvania south to the northern part of West Virginia but apparently it did not extend into Ohio. The beetles were more numerous in the western part of the Pennsylvania region than in the central part of the state. Professor F. N. Fagan,[2] State College, Pa., writes, " in the late summer of 1915 the cherry leaf-beetle made its appearance at State College . . . . They were not very injurious at that time, but in the summer of 1916 they became very plentiful and caused much damage to peach and cherry foliage."

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[1] Letter of April 3, 1916.

[2] Loc. cit., p. 944.

[3] Loc. cit., p. 21.

Regarding the abundance of the beetles in Michigan, Professor Pettit[3] writes that they were exceedingly numerous in the northern portion of the lower peninsula. They also occurred as far south as Port Huron. His correspondence records the insect at Alden, Bear Lake, Bellaire, Beulah, Central Lake, Cheboygan, East Jordan, Evart, Harbor Springs, Kingsley, Leetsville, Pellston, Thompsonville, Traverse City, Williamsburg and Wolverine (see Fig. 15). This outbreak was not a continuation of the invasion of the Alleghany area but doubtless similar conditions prevailed in the two regions preceding the appearance of the beetles in superabundant numbers.

SOME FACTORS THAT FAVORED DISPERSION.

All the factors that were responsible for this great invasion are not known and it is difficult to separate causal factors from coincidental phenomena. Thruout the Alleghany Mountains and the plateaus forming the Piedmont regions to the north and west of the highest ridges much unarable land has been denuded of forests. On most of this land grazing and forest fires have prevented reforestation but it is covered with vegetation in which the less valuable, quick-growing shrubs and trees are dominant. The bird cherry is one of the species which becomes established under such conditions. (Plate XLII, fig. 1.) This tree[4] is also found in swales and on much of the unproductive and waste land about farms in the Alleghany plateau region of New York (Plate XLI, fig. 2; Plate XLII, fig. 2). Thus an abundance of essential food had been provided for the beetles previous to 1915 and there exists at the present time a vast feeding ground for this species. Under these conditions an abundance of cherry leaf-beetles developed during 1914. To what extent parasitic and predaceous enemies affected *cavicollis* is not known

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[1] Cushman and Isley, loc. cit., pp. 3–5.

[2] Letter of October 20, 1916.

[3] Letters of October 20 and 25, 1916.

[4] Regarding the establishment of bird cherry in abandoned farm land and in land denuded of forests see Bray, " The Development of Vegetation of New York State," pp. 162 and 177.

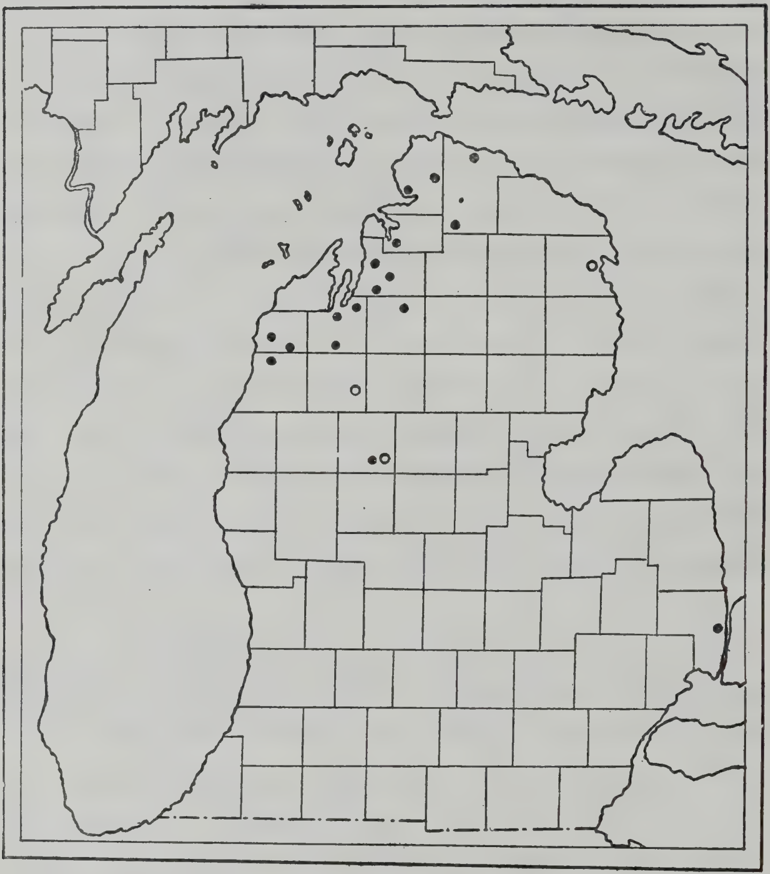

FIG. 15.— MAP OF THE LOWER PENINSULA OF MICHIGAN SHOWING INFESTATION BY THE CHERRY LEAF-BEETLE.

Black dots represent localities reported in 1915, circles show places reported during 1916. Localities furnished by Prof. R. H. Pettit.

nor do we know that a greater percentage of adults survived hibernation than normally. Several facts, however, are known: (1) There was a severe freeze over most of this area on May 27, 1915, which greatly reduced the amount of foliage on much of the vegetation, including the pin cherry. In this connection it is worthy to note that the outbreak of leaf-beetles at Ausable Forks, N. Y., in 1895 described by Dr. Lintner[1] occurred after a late frost. (2) Thruout the southern part of Chautauqua County tent caterpillars were abundant on the various species of wild cherry, including *P. pennsylvanica;* (3) the normal dispersion period of the species occurs in this region during the latter part of May and early June; (4) the beetles are more active when the temperature is above 60 deg. F., and they fly with the wind. On several such days just preceding and during the infestation the wind was southerly; and (5) the beetles were abundant on the pin cherry. The combination of these five factors we believe to be largely responsible for the great movement of the beetles to cultivated trees in western Pennsylvania and New York. It is possible that there were enough beetles in their native habitat to have caused a moderate infestation had there been no frost and no tent caterpillars, but it seems plausible that a diminished food supply would induce a greater percentage of beetles to migrate than would ordinarily abandon their native haunts where the means of subsistence were ample.

With respect to conditions in Michigan, Professor Pettit[2] says: " The beetle is a northern pest, which is very common on pin cherries about Lake Superior. There it is abundant each year." The freeze of May 27, extended thruout the northern part of Michigan.

During 1916 the attacks by the beetle were very slight in both regions with a few exceptions; in fact, so few beetles appeared thruout much of the area infested the previous year that it was not necessary to spray in order to protect cherry orchards. On the other hand tent caterpillars were not abundant during 1916 and there was no late freeze to damage the foliage; and yet, withal, a moderate infestation of the beetle appeared thruout Chautauqua County and in other sections of western New York. This was partially due to the scattered growth of pin cherry becoming more heavily infested during 1915; from which the progeny of these beetles migrated to cultivated trees during the spring of 1916. In spite of the behavior of the insect during 1916 it seems not unreasonable to consider the destruction of foliage by frost as one important factor in influencing the appearance of destructive numbers of the leaf-beetle during the previous year in both the Michigan and Alleghany regions.

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[1] Rept. of N. Y. State Entomologist for 1895, pp. 197-198.

[2] Letter of October 20, 1916.

ACTIVITIES OF LEAF-BEETLE DURING 1916.

During 1916 all reports in New York, with the possible exception of two, showed that the beetles were less numerous than during the preceding summer. They were observed thruout all the State west of the Genesee River, and in many localities in Chautauqua, Cattaraugus, Erie and Niagara counties they were also sufficiently numerous and destructive to influence growers to resort to spraying. At Conewango Valley some damage to young cherry trees was observed. Mr. C. L. Blood [1] of Jamestown reported that in his vicinity the beetles destroyed much foliage of the younger trees. Mr. Gideon C. Gorsline [2] writing from Cattaraugus County says " the red cherry beetle is very numerous in the village of Randolph and some of the outlying districts." Miss Cora Freeman [3] reported this pest as numerous about Angola. Mr. O. H. Houenstein [4] stated that similar conditions existed about Eden, and Mr. Otto A. Wagner [5] found the insects numerous at Alden. These correspondents agree that leaf-beetles were not as numerous during 1916 as during the preceding year. Mr. L. F. Strickland,[6] State Horticultural Inspector, reported that *cavicollis* was very numerous about Lockport during June, 1916, but was less abundant than in 1915. He also mentioned it as destructive to cherry stocks in a nursery at Batavia. Mr. Harvey E. Bolton [7] found the beetles quite scarce in cherry and peach orchards in the vicinity of Batavia. Mr. G. A. Blanchard [8] stated that the insects were very numerous in Middleport, but scarce in peach and cherry orchards in the surrounding country. Mr. A. B. Buchholz,[9] State Horticultural

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[1] Letter of June 21, 1916.
[2] Letter of June 22, 1916.
[3] Letter of June 29, 1916.
[4] Letter of June 23, 1916.
[5] Letter of June 27, 1916.
[6] Letter of June 27, 1916.
[7] Letter of June 24, 1916.
[8] Letter of June 28, 1916.
[9] Letter of June 26, 1916.

Inspector, wrote regarding the infestation in Orleans County during the past season: "On trees where the insect was very abundant last year, only a few can be found this season . . . . . . They are not sufficiently abundant to attract attention unless one is especially interested in the species." Mr. L. D. Rhind,[1] State Horticultural Inspector, and The George A. Sweet Nursery Company,[2] noted the insect as being scarce in the vicinity of Dansville during 1916. The region east of the Genesee River together with Orleans and Monroe Counties had a much lighter infestation by *cavicollis* during 1916 than did the region west of this river.

The infestation of this year was not due to migration from the usual habitats of this beetle, but was largely, if not entirely, owing to the development of an unusual number of the insects during 1915 on the scattered trees of *P. pennsylvanica* which grow on the waste areas in the vicinity of farms thruout western New York. The insect is normally very scarce on these trees, which perhaps may be accounted for by the unfavorable hibernating conditions that generally exist under trees in such locations. During the winter of 1915–16, many adults, however, hibernated safely and later migrated to cultivated cherries.

Thruout northwestern and western Pennsylvania the leaf beetle was present in numbers sufficient to cause some damage during 1916 but much less than the previous season. In Center County, Pa., however, there appears to have been a greater invasion during this season than in 1915. Professor F. N. Fagan[3] writes: "They were not very numerous during 1915, but during the following summer they became very plentiful and did much damage to peach and cherry foliage. From the numerous reports that came from the nearby territory I assume that the outbreak was quite general in Center County at least." Professor Pettit[4] writes that in Michigan the infestation during 1916 was much diminished in comparison with that of the preceding summer. His correspondence revealed the presence of the beetles in three localities: Alpena, Cadillac and Evart. The distribution of the insects during this season is shown in Fig. 15.

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[1] Letter of June 22, 1916.

[2] Letter of June 21, 1916.

[3] Letter of October 20, 1916.

[4] Letter of October 20, 1916.

APPEARANCE OF SPECIES DURING 1917.

In marked contrast to the two preceding years, there were very few beetles during 1917. Repeated examination of bird cherry growing along fences failed to reveal a single beetle. Several beetles were found in a natural habitat where *P. pennsylvanica* grew abundantly. This was on the higher land of the Alleghany Plateau region of Chautauqua County. Even under such favorable conditions of food and shelter we were unable to secure a sufficient number of specimens to continue insectary studies. Only a few letters were received from fruit growers during 1917 reporting the beetles as feeding on cultivated cherry or peach trees. In other words, the insect had diminished from a state of superabundance during 1915 to normal, perhaps subnormal, numbers by the third season following the outbreak.

DESCRIPTIONS OF LIFE STAGES.

Egg.— The eggs of the cherry leaf-beetle are small, yellowish, ellipsoidal bodies, and they vary in color with the amount of moisture on them; those in dry situations are stramineous while those that are moist appear bright yellow. The surface is finely reticulated, each unit of the network being irregularly hexagonal. (Plate XXXIX, fig. 1).

TABLE I.— MEASUREMENTS AND RANGE OF VARIATION OF EGGS OF THE CHERRY LEAF-BEETLE, FREDONIA, N. Y., 1916.

	Maximum.	Minimum.	Mean.	Standard deviation.	Coefficient of variability.
Length of egg	.896mm.	.608mm.	.782±.0024mm.	.0556±.0017mm.	7.1±.22
Breadth of egg	.777mm.	.507mm.	.695±.0023mm.	.0534±.0016mm.	7.7±.23
Length-breadth index	100	74	88.75±.18	4.12±.13	4.6±.14
Coefficient of correlation between length and breadth (r)					.805±.015
Number of eggs measured					247

The eggs show considerable variation in length and diameter as shown in Table I, which summarizes the measurements of two hundred forty-seven eggs that represent a random sample. From this data there were calculated the mean, standard deviation and coefficient of variability of the length and the breadth as well as the coefficient of correlation between the length and breadth. It was observed that a few eggs were almost perfect spheroids while others showed considerable difference between the two diameters. To determine the range of this variation a length-breadth index[1] was calculated by dividing the breadth by the length and multiplying the result by 100.

The measurements were made with a lens combination of a compound microscope whereby one space of the micrometer scale, equaled .0169 mm., but owing to the convexity of the eggs the determinations involve an error of about .005 mm.; therefore the mid-values are not given beyond the third decimal. In order that the data may be accessible to other workers the correlation table (Table II) follows:

TABLE II.—SHOWING THE CORRELATION OF EGG LENGTH AND EGG BREADTH.

Mid-values.	Egg Length																		
	.608	.625	.642	.659	.676	.693	.710	.727	.744	.761	.777	.794	.811	.828	.845	.862	.879	.896	
Egg Breadth. mm.																			
.507		1	2																3
.524																			0
.541	1	1																	2
.558						1	1												2
.575				1	1	1	1												4
.592			1	2	1	2	1	1		1									9
.608			1		3		1	1											6
.625							2	1	1						1				5
.642				1		1	3	2	2	2				1					12
.659							1	3	3	4	1								12
.676							1	2	4	7	4	5	1						24
.693							1	3	5	4	9	6	1	1	3				33
.710									2	4	9	9	6	4	5	1	1		41
.727								2	1	4	2	9	6	6	6	2		2	40
.744											2	7	3	12	6	3			33
.761											2	1	3	3	2	2	1	1	15
.777												2			2	1	1		6
	1	2	4	4	5	5	12	15	18	26	29	39	20	27	25	9	3	3	247

Larva.— After each molt the color of the larva is olive and the head and prothorax are large compared with the body. The setæ over the body and on the head are much more prominent at the beginning of the instar than later but the number and arrangement are the same as in the description below. After several hours the larva becomes a dark brown with the dark patches very close together.

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[1] This ratio is employed in anthropomorphic measurements to determine the cephalic index. Pearl and Surface (Bul. 110, Pt. III, Bureau of Animal Industry, U. S. Dept. of Agr.) have used the length-breadth ratio in a study of variation of the egg of the domestic fowl.

The head, prothorax and ninth segment are black. The several instars are much alike except in size (Plate XXXIX, fig. 2).

At the end of each instar the larva is almost cylindrical and the ground color of the skin is yellow. (Fig. 16.) Numerous black and fuscous spots and patches are present. The head is nearly hemispherical, slightly less than one-half as broad as the prothorax, black and bears a number of setæ on the dorsal surface. The prothorax is not quite as wide as the meso- or metathorax, but is nearly the same width as the abdomen. The dorsal surface is covered with a continuous oval dark-brown plate with a lighter, very thin, median line. There is a round concave area on each side of the median line. The cephalic margin of this plate bears eight setæ with a larger seta in the cephalic-lateral angle. Two small setæ are situated slightly mesad of the lateral margin midway between the cephalic and caudal margins. Two setæ are on the caudal margin, one on either side of the body and about half the distance from the median line to the lateral margin. The lateral protuberance bears a lune-shaped brown area and from this there project laterally four setæ; several small hairs are present. On the ventral surface the color is yellow, and there is a trapezoidal brown patch in the center with a small oval brown patch cephalad of it. The coxæ are nearly surrounded by two dark patches more or less triangular in shape. The prothoracic legs are black, end in a tarsal claw and bear several setæ.

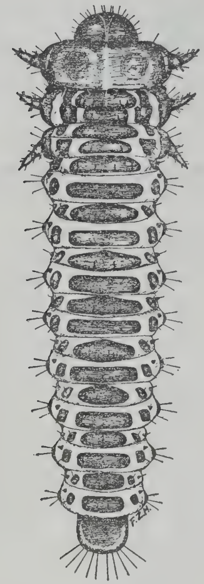

FIG. 16.—FULL GROWN LARVA OF THE CHERRY LEAF-BEETLE (ENLARGED).

The mesothorax and metathorax resemble each other except that sizes of the patches vary. The mesothorax is the broadest portion of the body. Two elongated patches extend across the median line of both of these segments. The cephalic one is the larger, and this on the mesothorax bears four setæ while the one on the metathorax has two setæ. The caudal patch in each case has only two setæ. Laterad of the patches are two dark spots, the caudal being the larger and bearing three setæ while the anterior one has a single seta. Laterad of these two spots is a large hemispherical protuberance bearing a large brown crescent-shaped area and four prominent setæ, the two nearer the center being larger. Just ventrad and slightly caudad of this protuberance is a smaller elongated area bearing two setæ while cephalad and ventrad is a protuberance; that on the mesothorax bearing a spiracle, and the one on the metathorax bearing a seta. Two small setæ are below the mesothoracic spiracle. On the ventral aspect of the meso- and metathorax there is an elongated trapezoidal patch in the center bearing two setæ, and caudad to this there are two small spots bearing one seta each. The legs are the same as on the prothorax.

All the abdominal segments except the ninth and tenth are similar in appearance. Each has two elongated dark patches similar to those on the metathorax; the anterior one bears four small setæ about equal distance apart on a line extending lengthwise thru the middle of the patch; while the posterior patch has two setæ near the ends. The posterior patch of the eighth segment bears four setæ. Laterad of each median patch is a spot bearing a seta. Laterad of each spot and somewhat cephalad is a smaller spot, the anterior one bearing a seta, the posterior spot bearing the spiracle. The lateral protuberances are tipped with large dark areas and bear three prominent setæ, the central one being the largest. Ventrally there is a median transverse trapezoidal patch bearing two setæ and laterad of this is a triangular spot bearing one seta; flanking this is an elliptical brown area bearing two setæ.

The ninth segment is U-shaped, dark brown and bears ten prominent setæ extending from the posterior margin. On the ventral aspect of the ninth segment is an elongated brown patch, and caudad of this is a crescent-shaped protuberance which serves as a foot, to the rear of which is the small tenth segment bearing the anus. The ninth segment and the head do not change in size during the duration of the instar, increase in size taking place at the moult before the parts become heavily chitinized. The measurements of the different instars are as follows:

First instar.— During the first larval instar the diameter of the head averaged .38 mm. with a range in individuals of from .36–.39 mm. The length of the entire insect was found to vary from 1.6–3.3 mm.

Second instar.— The head diameter averaged .54 mm.; individuals varying from .52–.57 mm. The length of the body varied from 2.3–4.5 mm.

Third instar.— The head diameter averaged .76 mm.; individuals varied from .72–.78 mm. The length of the body varied from 3.6–7.2 mm.

Pupa.— The pupa varies from 4 to 5 mm. in length and is bright yellow except the spines and most of the spiracles which are a dark brown (Plate XXXIX, fig. 3). There are no traces of the patches and spots so characteristic of the larva. The abdomen possesses considerable freedom of motion. The head bears well formed eyes, antennæ and mouth parts.

The antennæ show the segments plainly and are curved under the prothoracic and mesothoracic legs, the tips being just caudad of the ends of the tibiæ of the latter. The labrum bears two setæ, one near each lateral angle. The mandibles lie laterad of the labrum. The maxillæ show just below the mandibles. The vertex bears four setæ, one situated mesad of each eye and another mesad and caudad of this seta. On the posterior portion of the pronotum are eight setæ arranged in the shape of a V with the vertex pointing cephalad on the median line. Four setæ are on the anterior margin and a prominent seta in each anterior-lateral angle and another just caudad of the latter. Four small setæ are present on the mesonotum, two on either side of the median line. Four small setæ occur on the metanotum. The mesothorax bears a spiracle. The pro- and mesothoracic legs are folded across the body with tarsi lengthwise of the body. The metathoracic legs are folded under the wings. The tarsi of the prothoracic legs extend to the middle of the third abdominal segment, those of the mesothoracic legs to the middle of the fifth abdominal segment and those of the metathoracic legs to the seventh abdominal segment. Each leg has two prominent setæ on the dorsal aspect of the distal end of the femur. The wings are folded about the body, the elytra extending to the sixth abdominal segment, the metathoracic wings being under these, the tips showing near the legs.

The first six segments of the abdomen resemble each other. Each has a prominent seta on each side of the median line and one on each lateral protuberance. There is a prominent dark spiracle with tracheae showing through the body wall on the first five abdominal segments, mesad of the lateral protuberance and cephalad of the middle of each segment. The spiracles of the sixth and seventh segments are paler but similarly located. The eighth segment is compressed and bears four small setæ. The ninth segment bears two heavy spines each flanked laterally by a seta. The tenth segment bearing the anus is on the ventral aspect of the body and is very small and almost circular.

Adult.— In the northern portion of the range of this species the adult beetles are readily distinguished by their red color and their size (Plate XXXIX, fig. 4). The antennæ are black while the legs range in color from black to red. The insects are between 4.5

and 5.5 mm. (.17 and .21 inches) in length. The southern area of the normal habitat of the species overlaps the range of *rufosanguinea* which insect it closely resembles. The adult was briefly characterized by Le Conte,[1] and was later described with considerable detail by Horn [2] as follows:

> *G. cavicollis* Lec., oval, narrower in front, subdepressed; color dull red, slightly shining, very sparsely finely pubescent. Antennæ entirely black. Head red, coarsely punctured, without median depression, frontal tubercles smooth. Thorax nearly twice as wide as long, narrower in front, sides arcuate, or obtusely subangulate, hind angles distinct, base on each side obliquely sinuate, disc feebly convex, a broad depression on each side and another along the middle, surface coarsely punctured, more densely in the depressions; scutellum red. Elytra broader behind the middle, sides arcuate, margin explanate, humeri distinct, but rounded; sutural angle well marked, but obtuse; disc with coarse and deep punctures not crowded, less deep near the apex, interspaces smooth, shining. Body beneath red, the metasternum often piceous, sparsely finely punctate and finely pubescent. Legs variable in color entirely red to almost entirely piceous. Length .18–.22 inch (4.5–5.5 mm.).
>
> *Male.*— Claws finely bifid at apex. Last ventral segment broadly emarginate at apex, with a deep triangular depression limited by a sharply elevated line.
>
> *Female.*— Claws more deeply bifid, the parts more divergent. Last ventral segment with a very slight emargination, in front of which is a slight fovea.
>
> The middle coxæ are absolutely contiguous, the mesosternum is not prolonged between them. Except as to the color of the legs no variation has been observed in this species.
>
> *G. sanguinea* of Europe has been referred to as probably allied to this species, but an examination of that shows that it should be associated with *americana*, by reason of its convex form and not explanate elytral margin.

SEASONAL HISTORY.

EMERGENCE OF ADULTS FROM PUPAL STAGE.

In western New York most of the insects emerge as adults during the month of August of a normal year, altho some appear during the latter part of July and during early September. In 1915 Cushman [3] and Isley found the beetles emerging in their cages between August 23 and September 18 and during this period of twenty-six days the numbers that appeared each day were rather uniform. In 1916 the adults emerged at Fredonia from July 31 to September 2, covering a period of thirty-four days, and about seventy per ct. of the beetles emerged during the first ten days. The weather for this interval was more nearly normal than for the same months during the preceding year. Variation in seasonal conditions is without question largely responsible for the differences in dates of emergence during these two years. Also the methods employed in rearing the insects would unquestionably account, at least in part, for some of the variances reported in the foregoing observations.

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[1] Le Conte, J. L. Proc. Acad. Nat. Sci., Phila., p. 216. 1865.

G. cavicollis, obscure sanguineo-rufa, ovata convexa, thorace nitido, brevi, lateribus subangulatis, angulis omnibus prominulis, basi utrimque profunde oblinquo sinuato, disco cribratim punctato, profunde canaliculato, et utrimque late excavato, elytris parce brevissime pubescentibus sat dense cribratim punctatis; antennis nigris, tarsis fuscis. Long. .21.

Translation.— *G. cavicollis*, dark blood-red, oval convex, thorax shining, short, with sides subangular, all angles prominent, bases on both sides with deep oblique curve, disc punctated in the manner of a sieve, deeply channelled and broadly excavated on both sides, elytra sparingly covered with short hair rather densely punctated with pits; antennæ black, tarsi fuscous. Length .21 (inch).

[2] Horn, G. H. *Trans. Am. Ent. Soc.* 20 : 77, 1893.

[3] Loc. cit. pp. 14-18.

COLORATION CHANGES.

When the adult first emerges from the soil it is of a uniform yellow changing shortly to a yellow-brown color. After feeding it gradually assumes a darker tint and twenty-four hours after emergence the beetle presents a rather dark brown appearance. About the second day, and sometimes not until the third day, a distinct red tinge appears in the elytra and portions of the body. The rate of change in coloration is more rapid on warm than on cool days but during 1916 the minimum time required by males and females to acquire the full blood-red color of the normal adult was three days, while the maximum length was ten days and the mean six days.

FEEDING HABITS AFTER EMERGENCE.

The adults are rather sluggish and feed only slightly after their exit from the pupal cells. At no time during the fall was feeding as active as during the spring, and there was a tendency to seek hibernating quarters soon after emergence. The beetles crawled into the rubbish during cooler days and emerged from places of concealment and fed when the weather was warm.

HIBERNATION.

TIME OF ENTRANCE INTO HIBERNATION.

During the month of September the beetles confined in breeding cages showed a tendency to hibernate, but the complete disappearance of the insects into their winter quarters was a gradual process. On September 2, 1916, when the maximum temperature was 69 degrees F. and the sun shining brightly, nine beetles in one cage were feeding and twenty-eight beetles were in hiding in debris at the bottom of the cage. Five adults were added to this cage on September 9 and twelve beetles fed during the afternoon while thirty beetles hid in the rubbish. It is to be noted also that the day was clear with a maximum temperature of 74 degrees F. This ratio between the active and passive insects was maintained for nearly a week, during which time the weather was very warm. On September 22 when the maximum temperature was 67 degrees F. six beetles were on the leaves and thirty-six were concealed in rubbish. By October 1 all the beetles in the breeding cages were in hibernating quarters. In order to compare these results with conditions outside careful examinations were made during September of pin cherry foliage, in a locality where the beetles abounded during the summer, but in no instance were the insects detected. It appears from these observations that under normal conditions during 1916, entrance into winter quarters occurred earlier than in the cages.

The temperature records during and after this time of entrance into hibernation raise the question if, as with some other insects, low temperatures are largely responsible for the movement of the beetles to places of concealment for the winter. During the first half of October high temperatures prevailed and there was little rain until the 13th. During the first fifteen days of the month the maximum temperature was 83 degrees, the minimum 32 degrees, and the average mean temperature was 56 degrees. A killing frost occurred October 1 and this it is presumed exerted some influence in driving the beetles to shelter from which the warm weather of succeeding days was apparently not able to entice them.

CONDITIONS OF FOOD PLANTS AT TIME OF ENTRANCE INTO HIBERNATION.

During the month of September the pin cherry foliage was green and, while it perhaps was tougher than earlier in the season, to the eye it presented no distinct change which might be responsible for the cessation of feeding by the cherry leaf-beetles. The killing frost of October 1 did not destroy this foliage immediately but shortly afterwards the leaves began to show signs of decreased vigor. There was a distinct tendency to yellowing and the leaves dropped readily when touched. After the second killing frost on October 10 the trees and shrubs took on the autumnal tints and before many days practically all the foliage had fallen.

STAGES ENTERING HIBERNATION AND NATURE OF SHELTER.

As has already been suggested the adult is the only stage of the cherry leaf-beetle known to enter hibernation. All of the insects in the pupal stage as late as October 1 did not transform, and later died. In seeking quarters for hibernation the beetles crawled to the bases of the trees upon which they had been feeding and ultimately buried themselves in rubbish and leaf mold. The bird cherry generally grows in what ecologists designate as the forest margin association (Plates XLI and XLII). In such situations there are numerous bushes, brambles, weeds, and other vegetation which catch the leaves and other rubbish. As one penetrates deeper into the leaf mold this becomes more decomposed until finally there is the soil. It is this decomposed mold that shelters the beetles, and the nature of the shelter sought by the insects is clearly shown in Plate XLI, fig. 1, which was photographed a week previous to emergence in the spring. This location contained more than the average amount of leaf mold. In exposed places, as along fences and narrow strips of waste land, there is little opportunity for the formation of much leaf mold, and the absence of this may perhaps explain why the beetles do not normally infest the more isolated bird-cherry trees growing in such locations. A typical location for the hibernation of the beetles is illustrated in Plate XLII, fig. 1. Here beetles were observed emerging from winter quarters during the month of May in both 1916 and 1917.

EMERGENCE FROM HIBERNATION.

Time of emergence.— In 1916, the first emergence of the beetles from hibernation occurred in natural surroundings at Fredonia on May 27. Because of the backward season this date is believed to be somewhat later than the time of emergence during 1915. The first appearance of the beetles during 1917 at Lily Dale, eight miles from Fredonia and five hundred feet higher, was on May 30. The first adults emerged during two seasons about one week after the bird cherry was in full bloom.

Weather conditions preceding and during emergence.— Believing that the time of emergence from hibernation for any insect is a function of climatic conditions, records were kept during 1916 of the temperature, relative humidity, amount of rain and condition of the sky.

The temperature records were obtained by a thermograph which was carefully checked by a maximum and minimum thermometer in the regular United States Weather Bureau type of shelter. The relative humidity was secured by means of a sling psychrometer at 8 A. M., 1 P. M. and 8 P. M., and the arithmetical mean of the three readings was determined. Rainfall was measured by the United States Weather Bureau rain gauge and is given in hundredths of an inch. The character of the sky is given as cloudy if the sky averages .7 or more cloudy for the period from sunrise to sunset; clear if less than .3 cloudy, and partly cloudy if between .3 and .7 cloudy. These different conditions are represented by symbols used by cooperative observers:

○ clear ⊖ partly cloudy ⊕ cloudy.

These records from April 11 to May 30, 1916, are shown graphically in Fig. 17.

Similar records of the weather were kept during 1917 but the failure of the beetles to emerge in any of the places in the Lake Erie Valley where records were taken during the spring of 1916 makes it impossible to check the results of the previous season. Owing to the increased altitude, about 500 feet, of Lily Dale over that of Fredonia and the absence of records of the weather at the former place, comparisons are obviously impossible.

LENGTH OF THE HIBERNATION PERIOD.

If we assume October 1 as a normal time of entrance into winter shelter for this insect and the last week of May as the time of emergence, the length of the hibernating period in western New York is nearly eight months. It is possible that an early spring, such as occurred in 1915, might shorten this period to seven and a half months, but it is doubtful whether a combination of early fall and late spring would prolong this period over eight and a half months in this region. As the insect has not been studied in the southern part of its range we have no data regarding the length of this period there, but we would expect it to be shorter than that for western New York.

ACTIVITIES ON EMERGING FROM HIBERNATION.

The beetles upon emerging from their winter quarters are sluggish but they soon seek bird-cherry bushes and trees in search of food.

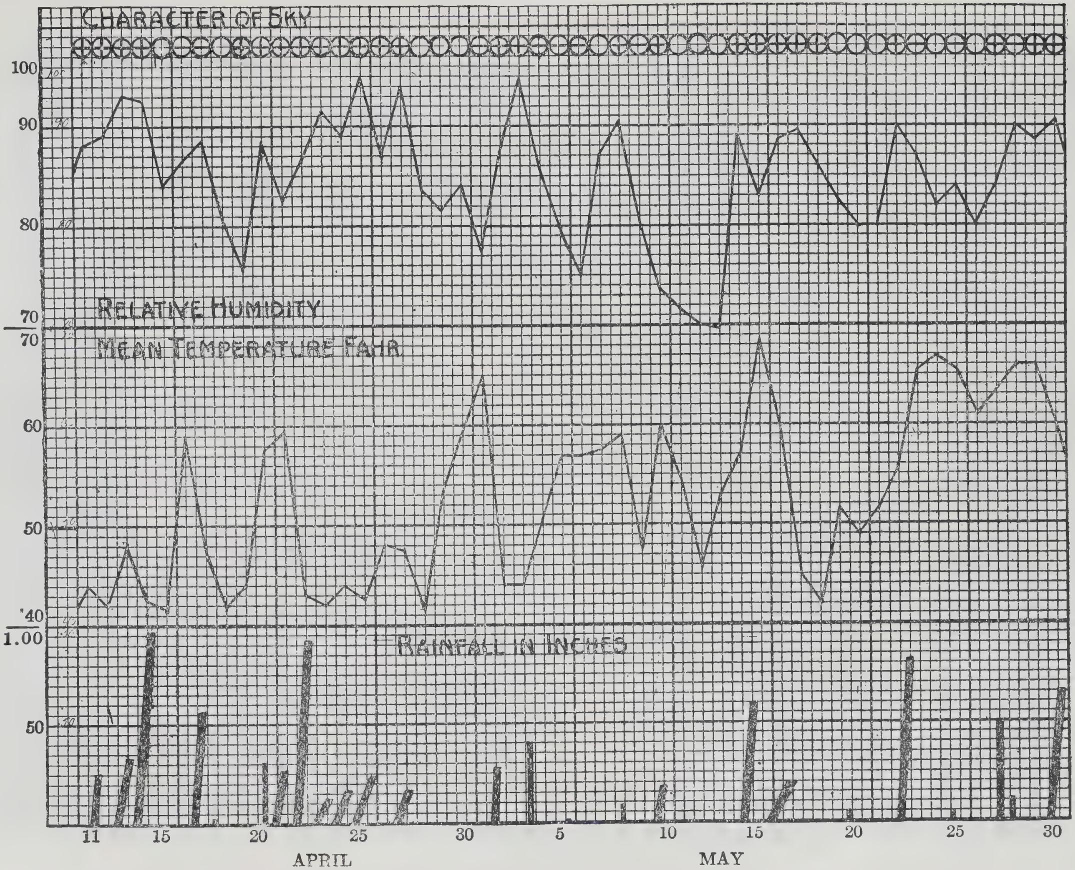

FIG. 17.—CHART SHOWING MEAN TEMPERATURE, RELATIVE HUMIDITY, CHARACTER OF THE SKY AND AMOUNT AND DURATION OF RAINFALL FOR EACH DAY FROM APRIL 11 TO MAY 30, 1916, AT FREDONIA, N. Y.

(Explanation in text, page 780.)

They seem to prefer younger trees of this species of cherry growing near their hibernating quarters. Feeding is not very active for several days, but thereafter if the temperature is high they feed rather voraciously, and if the weather is cool they crawl into sheltered places. Beetles were observed mating on the days when they emerged, at which time the temperature was 74 degrees F. and the sun was shining brightly.

FEEDING HABITS OF THE ADULTS DURING SPRING AND SUMMER

The nature of feeding by the adults varied to some extent according to the host. On bird cherry and young peach trees the insects attacked both the upper and lower surfaces of the leaves, showing a preference for the undersides. They ate irregular holes thru the leaves, avoiding the larger veins and the midrib. The initial holes in the leaf tissues were often enlarged by the feeding operations of several individuals. In spite of considerable damage the leaves of the pin cherry retained much of their original shape, altho when severely injured they presented a ragged appearance (Plates XLIII and XLIV, fig. 2). The outer areas of leaves that were badly affected died and curled, but such leaves adhered to the tree for some time owing to the green tissue which remained along the midrib. The wind usually tore such leaves into shreds. During the outbreak of 1915 evidence of the destructive work of the beetles was observed on every hand, and was very conspicuous after the leaves had turned brown. On account of the severity of injuries to foliage one could distinguish very quickly the bird cherry from other species of wild cherries even at considerable distances.

With large peach trees, however, there was much less concentration of the beetles, on account of the great mass of foliage; so that while holes were eaten in the leaves the foliage did not present the ragged appearance so characteristic of smaller trees of this fruit and more especially the bird cherry.

On sour cherries the beetles ate quite differently. They fed almost entirely on the undersides of the leaves, eating the softer tissue as far as the veins (Plate XLIV, fig. 1, and Plate XLV). Leaves that were severely attacked turned brown on the underside and showed a tendency to curl upwards, presenting a scorched appearance (Plate XLVI). Occasional leaves were entirely eaten thru and some feeding also occurred on the upper surfaces. After injured leaves had dried small areas dropped out, producing holes, but generally sour cherry foliage did not show the ragged appearance so typical of both the bird cherry and the peach.

In general, the insects obtained their sustenance from foliage, altho in several instances where trees were completely defoliated the pest attacked the green fruits of the sour cherry. At Jamestown one large sour cherry tree was observed on which practically all the fruit had been extensively pitted by the feeding of the beetles after the foliage had been destroyed. The tree was an isolated one and this form of injury was unusual. In commercial cherry orchards injuries to the young fruits were, in the main, unimportant.

The beetles also exhibited a partiality for the foliage of shoots growing about the bases of cherry trees or for seedlings in fence rows. In a number of instances the presence of such undergrowth served to protect the foliage of young trees from attack.

PROTECTIVE HABITS.

FEIGNING DEATH.

In common with many of the Chrysomelidæ the adults of *cavicollis* have the habit of dropping from the foliage and feigning death upon the approach of enemies. When the weather is cool the beetles do not fall to the ground so readily as when the temperature is above 60 degrees F. and if the weather is hot they are apt to fly instead of feigning death. During windy weather it requires considerable movement of the foliage to cause the beetles to drop, but during a calm the slightest movement of the leaves by any agency except the wind will cause practically every beetle to fall. Experiments with the creatures seem to indicate that they are able to distinguish between movements by the wind and those caused by other agencies. The beetles usually fall on their backs, remain motionless for a short time and, when danger appears to be past, turn over upon their ventral sides by raising their elytra sufficiently to allow their feet to grasp small objects, and right themselves. Feigning death evidently protects the beetles from birds and other predaceous enemies which seek living and moving creatures for food, for many forms of predatory animals refuse dead organisms in their diet and for this reason seldom touch motionless objects. This habit of the insect in dropping to the ground may be used, however, to good

EXPLANATION OF PLATES.

PLATE XXXIX.— LIFE STAGES OF THE CHERRY LEAF-BEETLE.

1. Eggs × 10; 2, larva × 6; 3, pupa × 8; 4, adult × 7.

PLATE XL.— LARVAE OF LEAF-BEETLE FEEDING ON THE FOLIAGE OF BIRD CHERRY.

Figs. 1 and 2 natural size; Fig. 3 enlarged.

PLATE XLI.— HABITAT OF HIBERNATING CHERRY LEAF-BEETLES.

1. Conditions about the bases of bird cherry trees shown in Fig. 2. The hibernating adults were found in the rubbish near the bases of these trees.
2. Cluster of trees of bird cherry growing at the edge of a field. Trees in bloom.

Both photos taken May 20, 1916, Fredonia, N. Y.

PLATE XLII.— VIEWS IN HABITATS OF THE BIRD CHERRY.

1. Bird cherry growing in cut-over land near Lily Dale, N. Y. This species is the dominant plant in many such areas. The other prominent vegetation is white pine, hemlock and blackberry.
2. Bird cherry growing in swale near cultivated land. The bird cherry is partially dominant here. Favorable conditions for the hibernation of the beetles.

PLATE XLIII.— EFFECTS OF FEEDING BY ADULT CHERRY LEAF-BEETLES ON FOLIAGE OF BIRD CHERRY.

PLATE XLIV.— EFFECTS OF FEEDING BY ADULTS OF CHERRY LEAF-BEETLE.

1. Characteristic feeding on the lower surface of a sour cherry leaf.
2. A common condition of the foliage of the bird cherry in Chautauqua County during 1915.

PLATE XLV.— EFFECTS OF FEEDING OF CHERRY LEAF-BEETLE ADULTS ON THE UPPER AND LOWER SURFACES OF LEAVES OF SOUR CHERRY.

This foliage was curled when taken from the tree but was expanded to show feeding. All injured portions were brown while uninjured parts were green.

PLATE XLVI.— CHARACTERISTIC CONDITION OF SOUR CHERRY FOLIAGE AFTER INJURY BY ADULTS OF CHERRY LEAF-BEETLE.

This foliage had a general brown appearance when photo was taken.

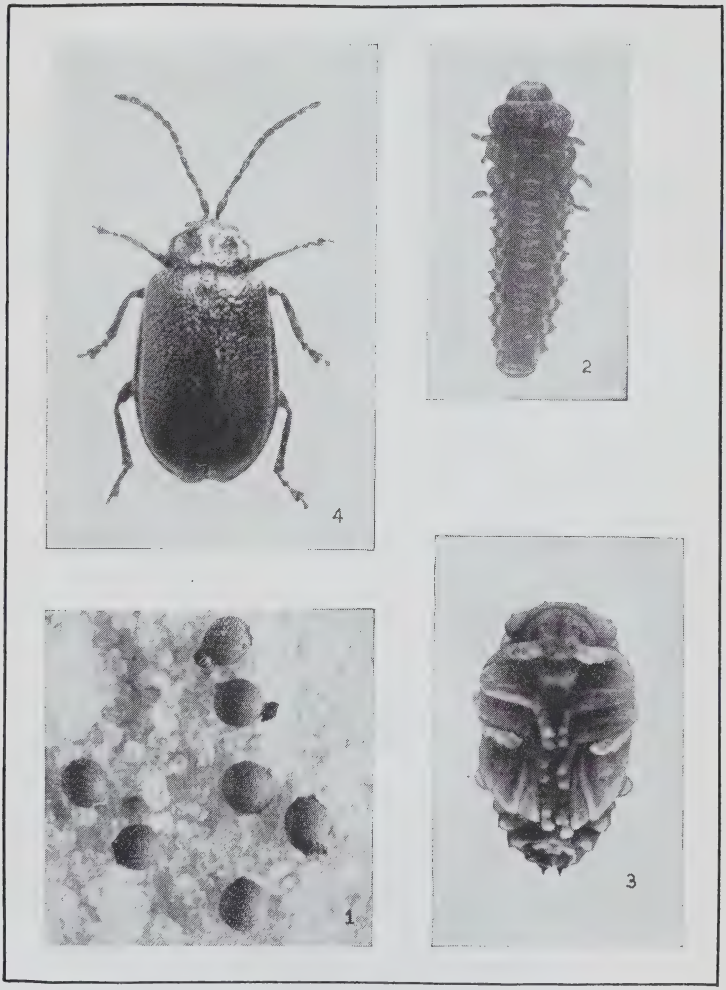

Plate XXXIX.— Life Stages of the Cherry Leaf-beetle.
1, Eggs × 10; 2, larva × 6; 3, pupa × 8; 4, adult × 7.

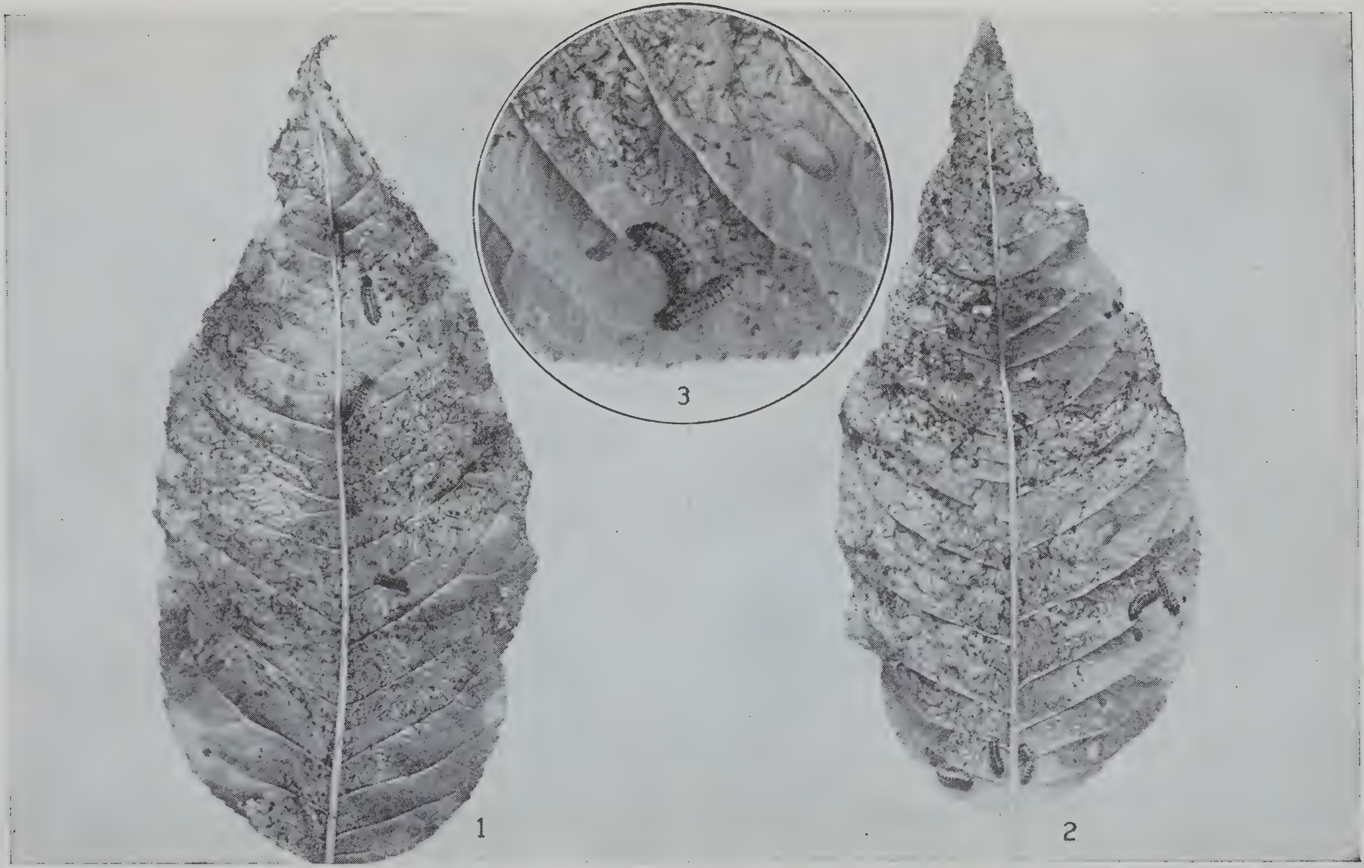

Plate XL.— Larvæ of Leaf-beetle Feeding on the Foliage of Bird Cherry.
Figs. 1 and 2 natural size; Fig. 3 enlarged.

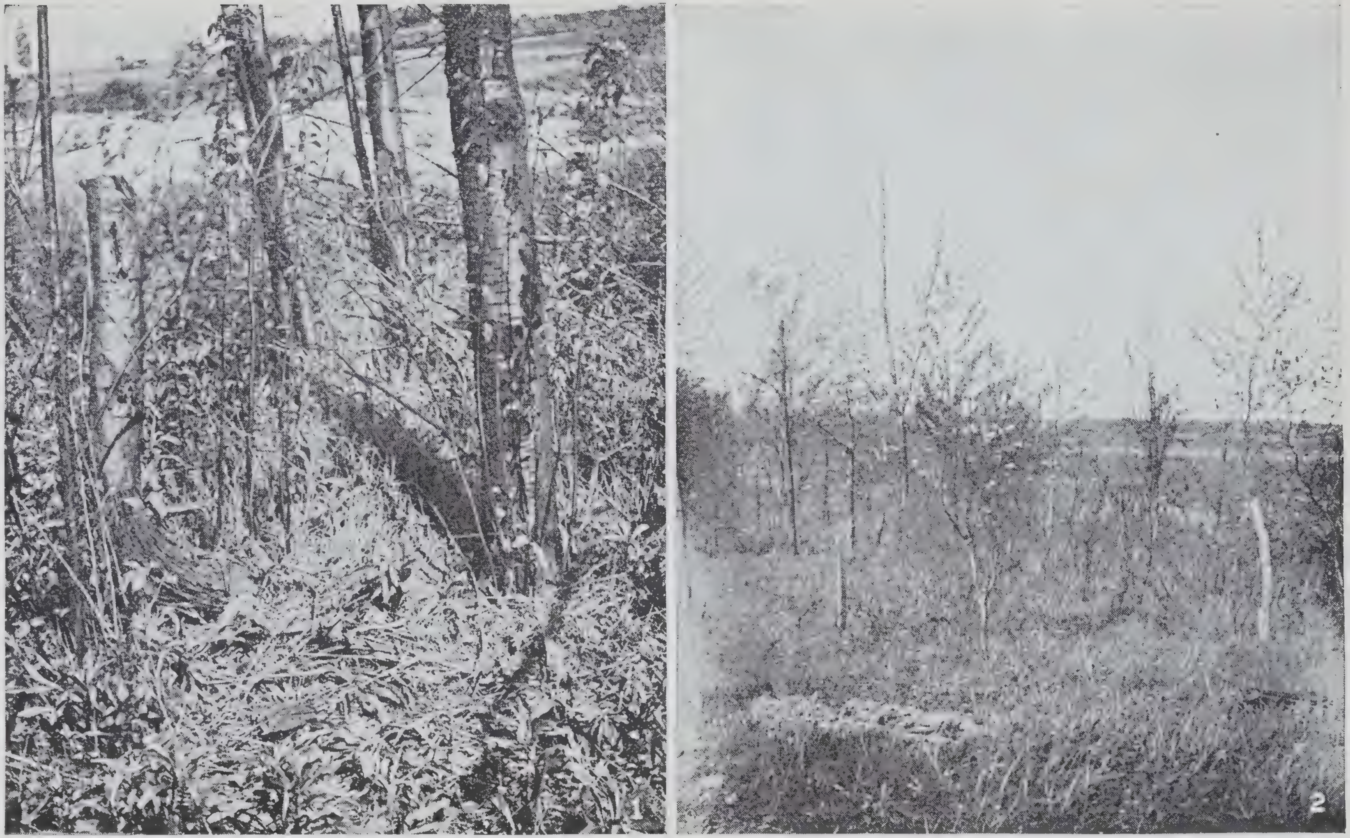

PLATE XLI.— HABITAT OF HIBERNATING CHERRY LEAF-BEETLES.

(See p. 518 for explanation.)

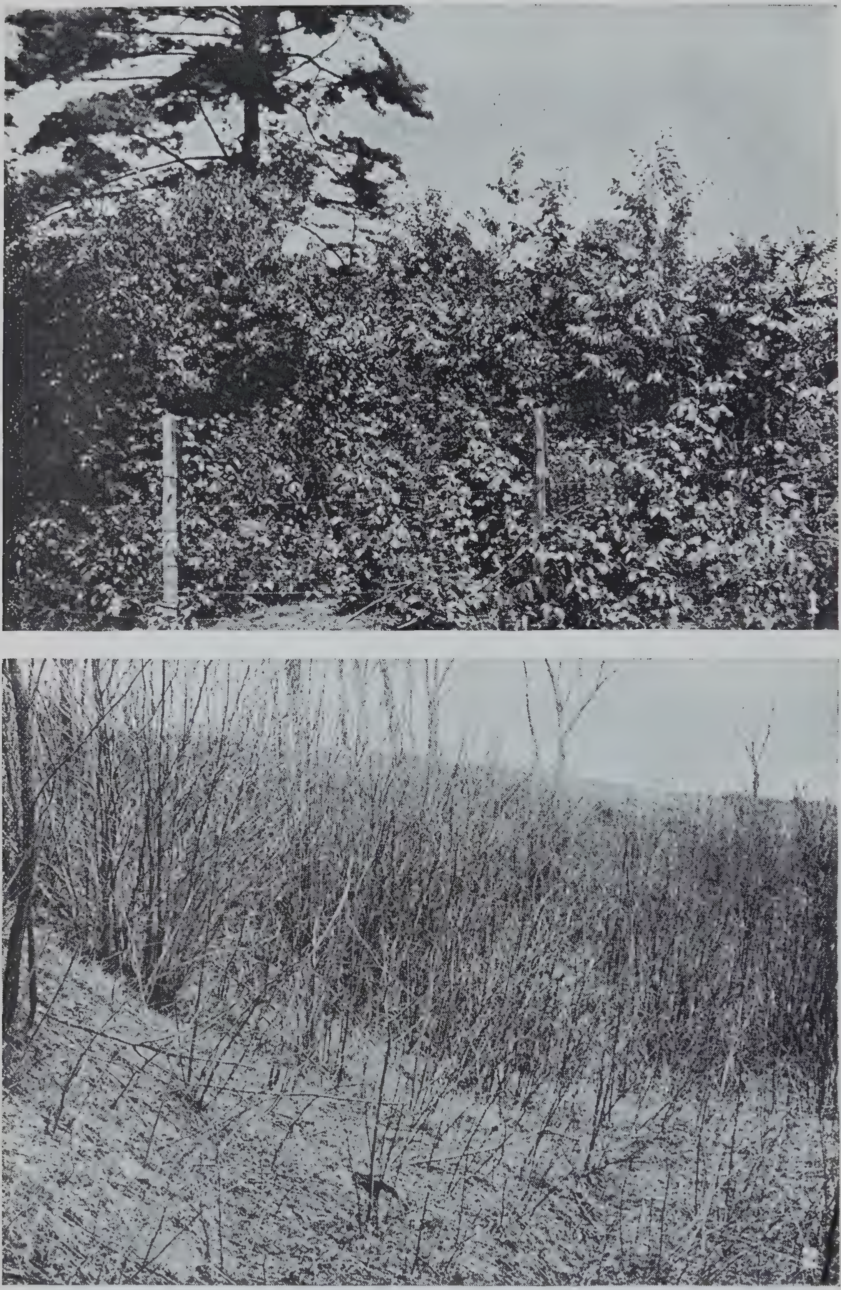

Plate XLII.— Views in Habitats of the Bird Cherry.
(See p. 518 for explanation.)

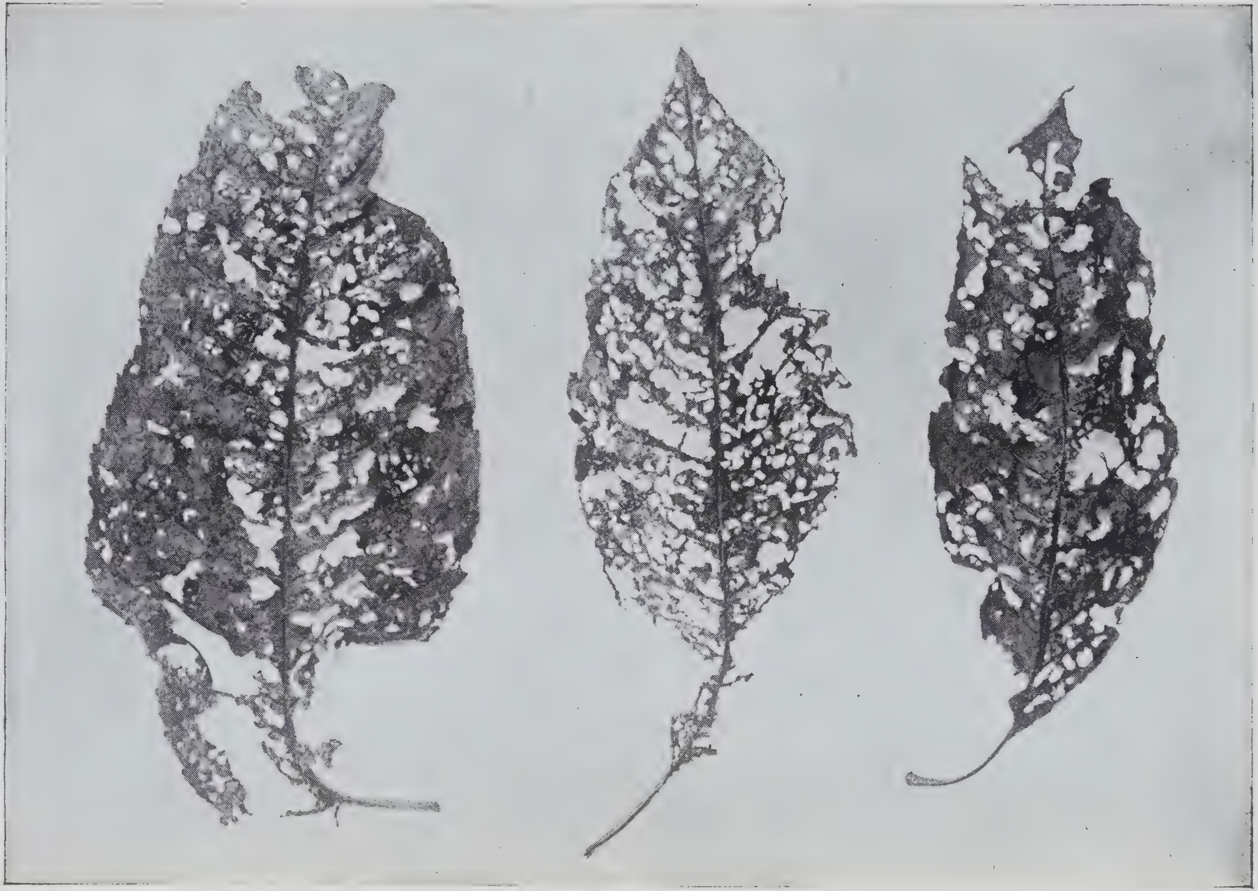

Plate XLIII.— Effects of Feeding by Adult Cherry Leaf-beetles on Foliage of Bird Cherry.

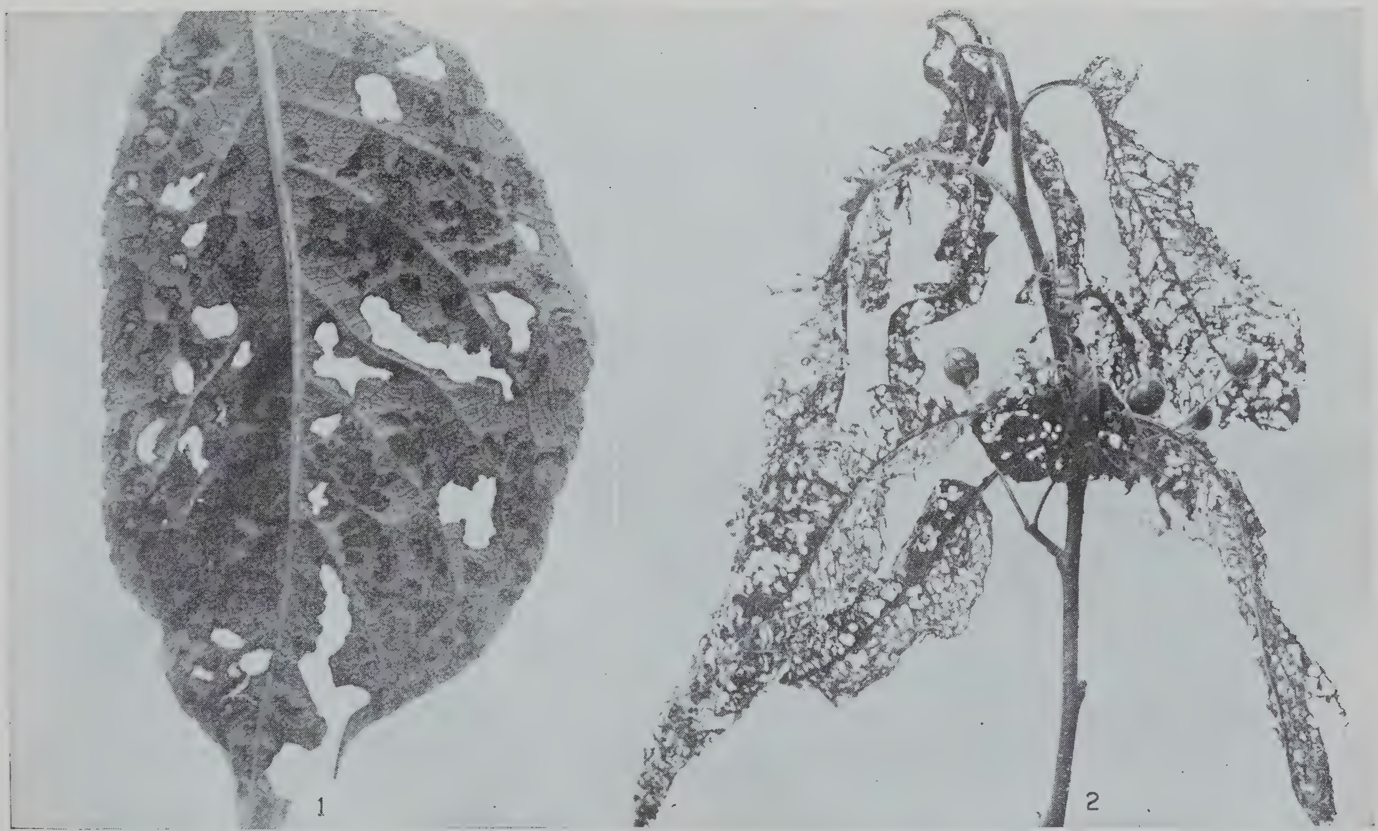

Plate XLIV.— Effects of Feeding by Adults of Cherry Leaf-beetle.
(See p. 518 for explanation.)

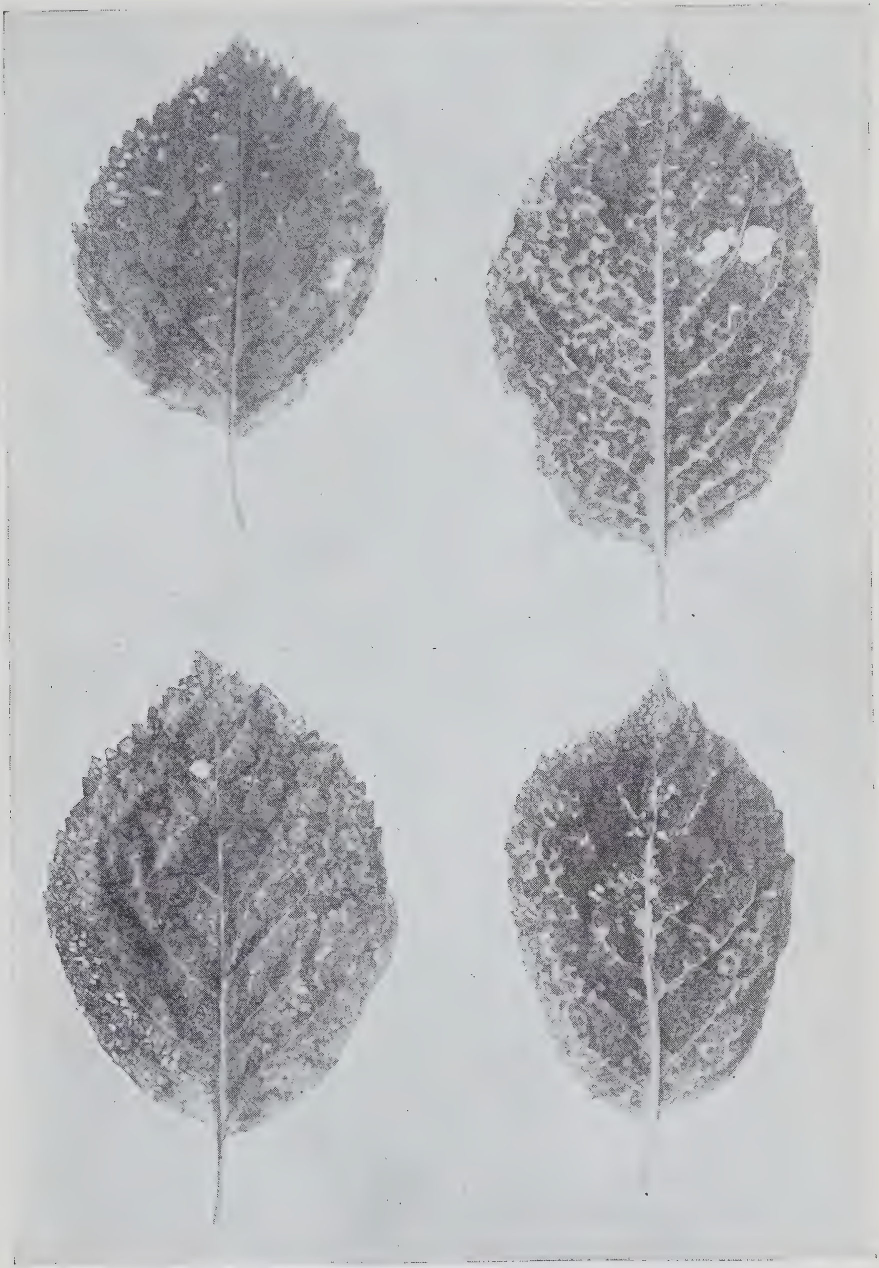

Plate XLV.— Effects of Feeding of Cherry Leaf-beetle Adults on the Upper and Lower Surfaces of Leaves of Sour Cherry.
(See p. 518 for explanation.)

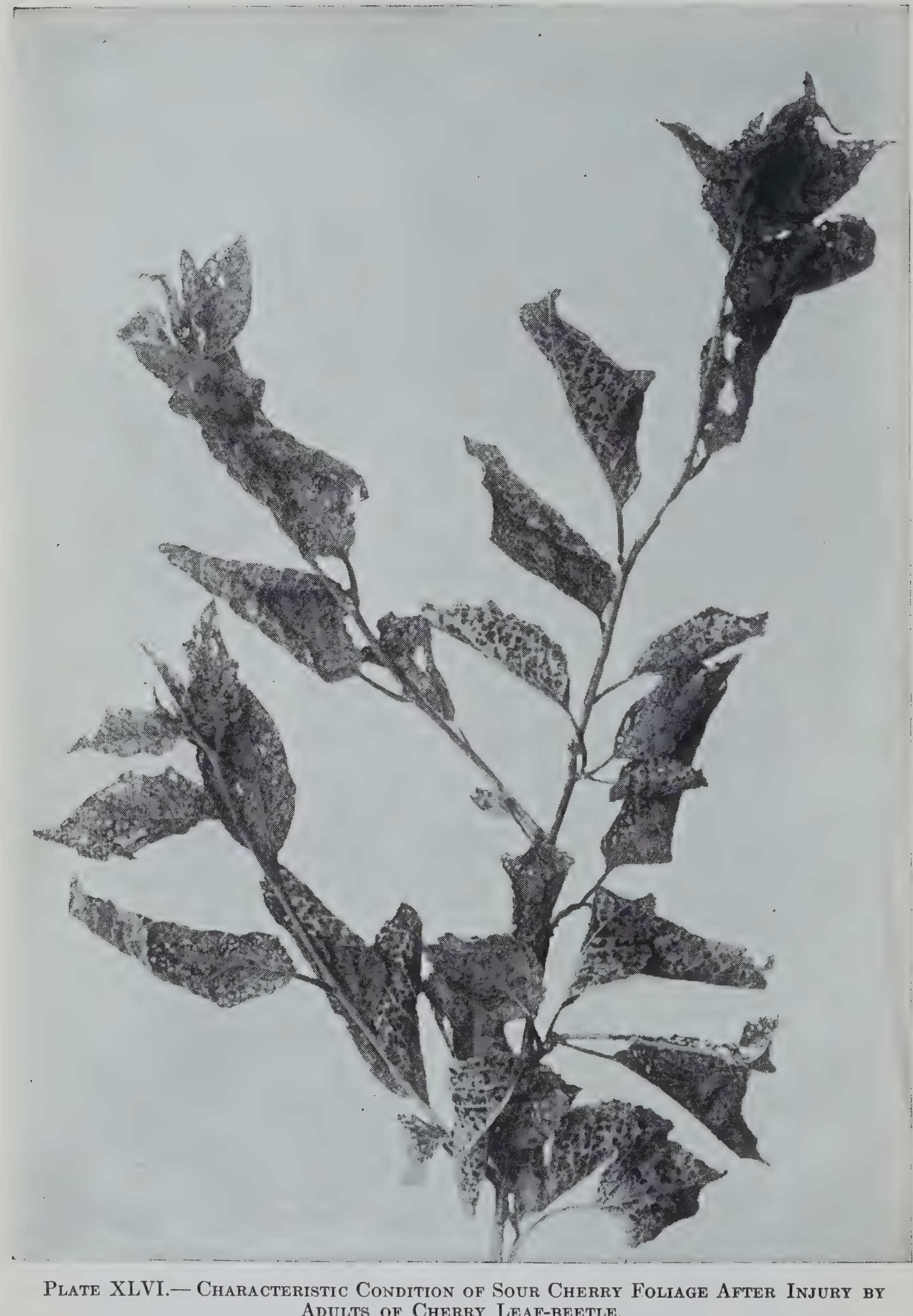

Plate XLVI.— Characteristic Condition of Sour Cherry Foliage After Injury by Adults of Cherry Leaf-beetle.
(See p. 518 for explanation.)

advantage by the fruit grower for the purpose of collecting the beetles, and thus an instinct which in its natural evironment serves for the protection of its possessor redounds to its disadvantage under orchard conditions.

PROCRYPTIC COLORATION.

By procryptic coloration is meant coloration which aids in concealment. As one sees these shiny, red beetles feeding on green foliage it would seem that their color must render them very conspicuous objects to their enemies; but as soon as the redcoats drop to the ground and lie inactive on their backs they become imperceptible and are very difficult to detect. The venter and legs are quite dark, in some specimens piceous, and they blend with the color of the leaf mold in their native haunts. The dissimulation is not as complete on lighter soil such as obtains in many orchards, especially during dry weather, but even here procryptic coloration is apparent and should be some advantage to the insect. Feigning death and procryptic coloration are supplementary protective factors, which collectively doubtless afford considerable protection to the beetle in its struggle for existence.

FEEDING ON THE UNDERSIDE OF THE FOLIAGE.

The tendency of the beetles to feed largely on the undersides of the leaves may also be classed as a protective factor since it renders them less visible to birds. Moreover it facilitates their dropping to ground with the jarring of the foliage, as when a bird alights on a twig. This habit does not render the beetle entirely immune to attacks by birds, as will be noted in the discussion of predaceous enemies, and there is no definite knowledge as to its influence on predaceous insects. On account of its feeding on the under surfaces of the leaves the pest is more difficult to control by poisons because of the care required to place properly the poisoned spray.

MATING HABITS.

TIME AND FREQUENCY OF MATING.

The beetles were observed mating the day after hibernation and the act was intermittent for a period of about a month and a half. The greatest activity occurred during the month of June and was always most marked on warmer days. Individual couples in cages were observed mating a number of times, six coitions being the maximum of one pair. However, as it was impossible to keep these cages under constant observation the actual number of copulations for each pair in confinement was not secured but was doubtless greater than that given. The time of each act was not long and the beetles during copulation were easily disturbed.

AGE AT BEGINNING OF COPULATION AND DURATION OF PERIOD.

Copulation was not observed among the beetles before the hibernation period, so all beetles must have been at least nine months from the time of hatching before reaching sexual maturity. The majority were nearly ten months of age at the beginning of this period. They continued to mate for nearly two months so the age at the end of this period would average about twelve months.

OVIPOSITION.

AGE OF BEETLES AT BEGINNING OF OVIPOSITION.

So far as is known no adult of this species deposits eggs until after passing the hibernating period, and moreover this function does not appear normally until after fertilization. Since egg laying occurs during the months of June, July and August, as will be explained later, the beetles are then never less than nine months from the time of hatching. Some individuals may be thirteen months of age at the time they deposit their last eggs.

PERIOD OF OVIPOSITION AND NUMBER OF EGGS LAID BY BEETLES.

In general the oviposition period extends from the first week in June until the middle of August, the maximum number of eggs being deposited during early July. In 1915 the first eggs were found at Fredonia on June 11, but as we were unacquainted with the eggs and especially the place of oviposition it is likely that the first eggs were laid several days earlier and not found. The first eggs discovered during 1916 were found under natural conditions on June 5. This was nine days after the first beetles were observed in coitu. For more than a week after emergence the beetles did not copulate freely so it was June 7 before we secured the first cages of copulating pairs to observe egg-laying habits and the first eggs were deposited in the cages on June 10.

Four copulating pairs of beetles were caged on June 7 and another pair on June 10; and on June 14 thirty-one additional couples taken from cherry foliage during mating were placed in individual cages. The cages consisted of glass jars of different sizes in which soil and leaf mold were placed to a depth of about one inch and careful attention was given to keep the soil conditions similar to those at the bases of cherry trees.

Foliage of the bird cherry was added to serve as food and a piece of growing bird-cherry wood was placed in the soil to imitate a growing tree, particular attention being paid to have a portion of the wood enveloped with foliage. Most of the eggs were placed on this bark just beneath the surface of the soil. It was found that the size of the cage had practically no effect on the egg laying but that the proper amount of moisture was an important factor. The cages of 1915 proved that the beetles are very fastidious regarding conditions for oviposition and that they will not lay eggs unless conditions are to their liking. Owing to the time required to find the eggs and the number of cages it was necessary to examine one-half, only, of the cages each day, hence the egg record is given in two-day periods. It was feared that unless several cages were used we would fail to secure results comparable to conditions in nature since in all life-history work individual variation is a factor that must be kept in mind. From these cages oviposition records were secured which are summarized in Tables III to VI inclusive.

Table III.— Egg-laying Period and Duration of Life of the Cherry Leaf-beetle at Fredonia, N. Y., during 1916.

	Days	Dates
Total egg-laying period for all females	66	June 5 to Aug. 9.
Longest egg-laying period for a single female	54	June 17 to Aug. 9.
Shortest egg-laying period for a single female	10	June 17 to June 26.
Average egg-laying period for a single female	28	
Maximum number of days after June 8th that female lived	66	Aug. 13.
Minimum number of days after June 8th that female lived	18	June 26.
Average number of days after June 8th that female lived	42	July 20.
Maximum number of days after June 8th that male lived	109	Sept. 25.
Minimum number of days after June 8th that male lived	21	June 29.
Average number of days after June 8th that male lived	41	July 19.

As will be noted in Table III, the egg-laying period for all females in the cages extended from June 9 to August 9 or about sixty-one days, and the total egg-laying period observed at Fredonia during 1916 was sixty-six days. After June 26 the number of females was gradually reduced by death until by August 9 only one female remained. In this respect the caged insects closely followed conditions in nature, barring accidental deaths. It was our aim to secure the copulating pairs on the same date but the sluggishness of the beetles due to rains and low temperatures for over a week prevented this. However, very few eggs were laid at the bases of cherry trees before June 15 and we believe that the results in the cages are a fair index of the number of eggs laid under outside conditions.

The number of eggs laid by a female beetle varied greatly in the thirty-six cages as Table IV shows.

TABLE IV.— NUMBER OF EGGS LAID BY THE CHERRY LEAF-BEETLE AT FREDONIA, N. Y., DURING 1916.

Largest number of eggs laid by a single female	294
Least number of eggs laid by a single female	10
Mean number of eggs laid by a single female	93±7
Standard deviation (eggs)	62.8±5.0
Coefficient of variability	67.8±5.5
Number of cages of paired beetles	36

The excessive variation in the number of eggs laid by a female as shown by the standard deviation, coefficient of variability and the high probable errors, illustrates the fastidiousness of the beetles as regards environment during egg laying. This variation occurred notwithstanding the fact that an effort was made to keep conditions the same in all the cages.

We are inclined to believe that the true mean number of eggs laid is somewhere between the average and the maximum given in the above table; for it is probable that the number of eggs secured in several of the cages is below that which the beetles would normally have deposited in nature. It would seem that in some of the cages with low records we failed to secure the proper conditions for egg-laying and therefore the beetles refused to deposit the normal number of eggs. If the records of five low cages are left out of consideration the mean would show one hundred five eggs per female.

The number of eggs deposited in all the cages during each period shows marked variation. Attention is called to Table V, which contains the egg-laying record of the insects under observation.

TABLE V.— NUMBER OF EGGS DEPOSITED BY ALL CAGED BEETLES FOR EACH TWO-DAY PERIOD. FREDONIA, N. Y., 1916.

Period	Number of eggs	Number of females	Period	Number of eggs	Number of females
June 9–10	6	4	July 11–12	209	31
June 11–12	6	5	July 13–14	199	28
June 13–14	6	5	July 15–16	152	23
June 15–16	21	36	July 17–18	108	19
June 17–18	75	36	July 19–20	92	19
June 19–20	129	36	July 21–22	43	15
June 21–22	20	36	July 23–24	31	13
June 23–24	75	36	July 25–26	30	9
June 25–26	111	36	July 27–28	43	8
June 27–28	308	35	July 29–30	22	7
June 29–30	237	35	July 31–Aug. 1	19	6
July 1–2	182	35	Aug. 2–3	12	5
July 3–4	366	35	Aug. 4–5	15	4
July 5–6	371	32	Aug. 6–7	1	1
July 7–8	254	31	Aug. 8–9	1	1
July 9–10	190	31			
Total				3,334	

The average number of eggs per female during the same period is given in Table VI. The meteorological conditions occurring during the period of egg-laying are shown in Fig. 18.

TABLE VI.— NUMBER OF EGGS LAID PER FEMALE CHERRY LEAF-BEETLE FOR EACH TWO-DAY PERIOD, FREDONIA, N. Y., 1916.

Periods.	Average number of eggs per female.	Periods.	Average number of eggs per female.
June 9–10	1.5	July 11–12	6.7
June 11–12	1.2	July 13–14	7.1
June 13–14	1.2	July 15–16	6.6
June 15–16	.6	July 17–18	5.7
June 17–18	2.1	July 19–20	4.8
June 19–20	3.6	July 21–22	2.9
June 21–22	.6	July 23–24	2.4
June 23–24	2.1	July 25–26	3.3
June 25–26	3.1	July 27–28	5.4
June 27–28	8.8	July 29–30	3.1
June 29–30	6.8	July 31–Aug. 1	3.1
July 1– 2	5.1	Aug. 2– 3	2.4
July 3– 4	10.2	Aug. 4– 5	3.8
July 5– 6	11.6	Aug. 6– 7	1.0
July 7– 8	8.2	Aug. 8– 9	1.0
July 9–10	6.1		

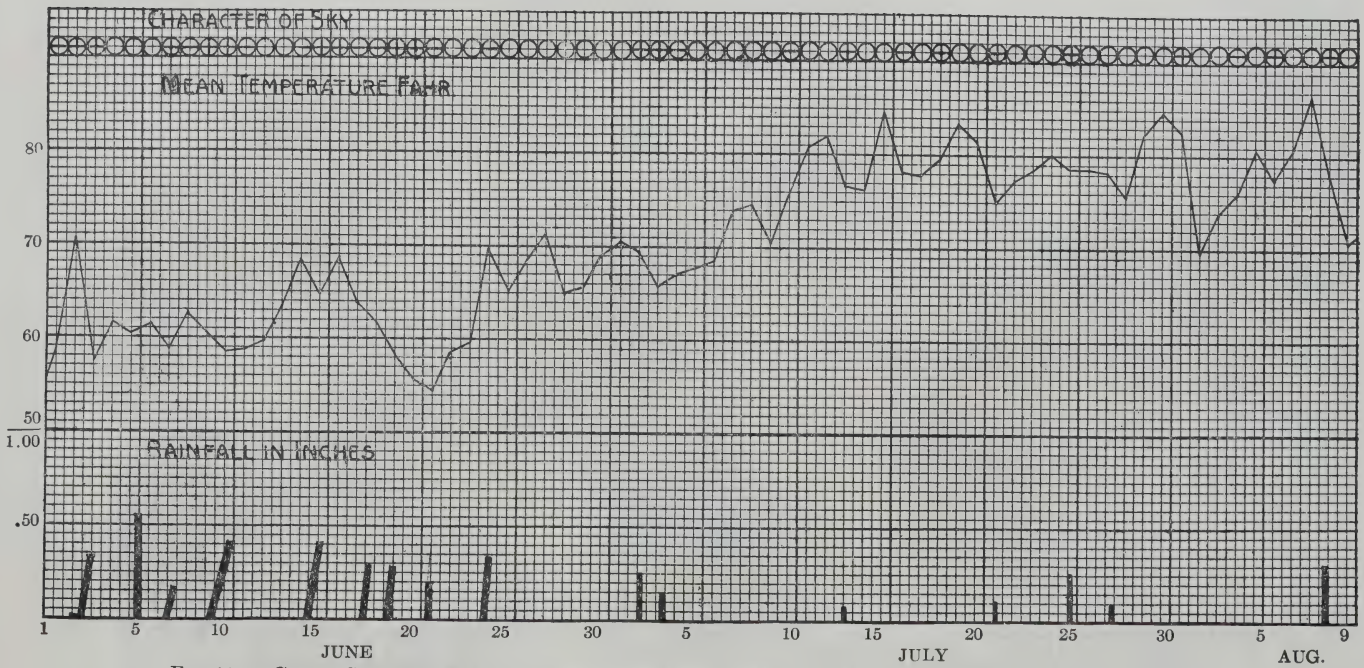

FIG. 18. — CHART SHOWING CLIMATIC CONDITIONS FROM JUNE 1 TO AUGUST 9, 1916, AT FREDONIA, N. Y.

PLACE OF EGG DEPOSITION.

Under natural conditions the majority of the eggs are laid in the ground at the bases of the trees upon which the insects are feeding. Most of them are placed in the soil just beneath the surface, altho a few are found to a depth of one inch, and some are deposited on top of the ground. They are often deposited loosely, but generally are attached by a gluish substance to rootlets, small stones and rubbish. It is therefore often difficult to remove the eggs without injuring them. In the breeding cages it was necessary to remove the eggs as they were counted, and as a result many were injured so that only a small percentage hatched.

During the course of our observations eggs were generally found glued to the bases of the trees from a depth of about an inch beneath the soil to a height of several inches above the surface. An exception to this habit was observed in a cherry orchard at Westfield where eggs were detected over all portions of the trunks as high as the forks of the limbs, or as much as three feet above the ground.

Moisture appears to be the controlling factor in the selection of a suitable place for oviposition by the females and so during periods of ordinary weather the females place the eggs in the damp soil. However, during rainy periods the bases of the trees are damp and are apparently attractive to the beetles for purposes of egg laying. In the cherry orchard at Westfield the trees were headed low so that the trunks were well shaded and remained moist for a considerable period. Besides, rainy and cloudy weather prevailed from June 14 to June 21, during which time these trees were continuously wet. By reason of these conditions beetles apparently were induced to lay eggs in what appeared to be unnatural situations.

PROTECTION OF EGGS.

The deposition of the eggs in the soil would naturally protect them from many predaceous enemies, but the chief protection is doubtless against unfavorable physical conditions, such as dryness and heat from the direct rays of the sun. It is believed that the latter is the more important factor because of the fact that no destruction of the eggs, by either predaceous or parasitic enemies, was noted, even during 1915 when eggs were deposited in such abundance.

DURATION OF LIFE OF THE CHERRY LEAF-BEETLE.

The first larvæ of the cherry leaf-beetle were found on July 1, 1915, but the maximum number emerged during July and August. During 1916 the first larvæ appeared on June 23, while the majority of individuals hatched during July and the first half of August. In the cages a number of the hibernated beetles died during June but July was the month during which most of the beetles succumbed. The last female died August 13, but four males lived until the last week of September. The average life of the beetles therefore was about twelve and one-half months. Of the specimens displaying exceptional longevity, the greatest duration of life of the female was not over fourteen months, while the males may have survived for about fifteen months, unless they hatched later than the average during the preceding year, in which case their period of existence would be somewhat less than that mentioned.

THE EGG.

DURATION OF INCUBATION PERIOD.

In his observations of the species at Washington, D. C., during 1899 Chittenden[1] states that the first egg hatched on June 26, which was eleven days from the date of deposition. During July, 1915, Herrick[2] and Matheson at Ithaca obtained as the longest incubation period eighteen days, and the shortest period was fourteen days, with an average of sixteen days.

Owing to the difficulty of securing eggs from the beetles in the observation cages during 1915, it was found necessary in order to secure data on the length of the incubation period to remove eggs from the soil at the bases of certain trees, precautions being taken to obtain them shortly after they were deposited. These were transferred by means of a camel hair brush to a cage consisting of a Van Tieghem cell cemented with vaseline to an ordinary glass slide. On the bottom of the cell a few fibers of cotton were spread to hold the eggs and prevent their rolling; and a drop of water was placed in the cell occasionally to keep the air moist, reproducing as far as possible the conditions of moisture of the earth from which the eggs were obtained. A cover glass was used to enclose the cell.

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[1] U. S. Dept. Agr. Div. Ent. Bul. 19, n. s., p. 93. 1899.

[2] *Jour. Agr. Research*, 5:946–947. 1916.

Three lots were started with eggs which were laid on June 17 and one lot with eggs which were deposited on June 18, 1915. The records of hatching are summarized in Table VII.

Table VII.— Incubation of Eggs of Cherry Leaf-beetle at Fredonia, N. Y.

Record of eggs deposited on June 17, 1915. (Temperature records in degrees F.)											Total eggs hatched.
Duration of incubation period (days)	10	11	12	13	14	16	18	19	20	21	
Average daily mean temperature to day indicated....	65.9	65.6	65.6	66.2	66.3	66.6	66.5	66.4	66.1	66.1	
Lot 1, No. of eggs hatched........	1	0	9	0	0	1	5	6	0	3	25
Lot 2, No. of eggs hatched........	1	3	0	13	1	5	0	7	1		31
Lot 3, No. of eggs hatched........	2	0	0	0	0	0	2				4
(Record of eggs deposited on June 18, 1915.)											
Duration of incubation period (days)......................	12	13	15	18	19						
Average daily mean temperature..	66.4	66.5	66.8	66.9	66.6						
Lot 4, No. of eggs hatched.......	1	6	2	8	3						20
Longest incubation period..........................	21 days.										
Shortest incubation period..........................	10 days.										
Average..	15.8 days.										
Average mean temperature..........................	66.3 degrees F.										

By reason of the experience during the previous year it was possible to carry on more extensive observations on the egg stage during 1916. In the breeding cells eggs hatched over a period ranging from June 23 to August 1. These dates do not represent the entire period of hatching of eggs under normal conditions, which was somewhat greater; and they are of value only in connection with the particular eggs which were under observation. Notwithstanding the considerable number of larvæ that hatched, it should be stated there was high mortality among the eggs, which was largely ascribed to the methods of handling them. The records dealing with the incubation of three hundred thirty-nine eggs are given in Table VIII.

TABLE VIII.— INCUBATION OF EGGS OF CHERRY LEAF-BEETLE AT FREDONIA, N. Y., DURING 1916.

LOT NO.	Date of oviposition.	Date of hatching.	Number of eggs hatched.	Length of egg stage.	Average mean temperature for period.
				Days	*Degrees F.*
1	June 5	June 23	5	18	61.1
	June 5	June 25	5	20	60.9
	June 5	June 28	2	23	61.9
2	June 19	July 4	25	15	65.0
3	June 21	July 5	9	14	66.3
4	June 25	July 14	7	19	70.9
	June 25	July 16	32	21	71.8
5	July 3	July 13	6	10	72.7
	July 3	July 14	15	11	73.0
	July 3	July 16	50	13	74.2
	July 3	July 17	38	14	74.4
	July 3	July 19	2	16	75.0
6	July 8	July 19	3	11	77.9
	July 8	July 22	1	14	78.3
7	July 10	July 19	3	9	79.0
	July 10	July 22	12	12	79.3
	July 10	July 23	1	13	79.1
8	July 18	July 27	38	9	79.3
	July 18	July 28	40	10	79.2
	July 18	July 29	23	11	78.8
	July 18	July 30	21	12	79.1
	July 18	Aug. 1	1	14	79.8

Maximum number of days required for incubation	23
Minimum number of days required for incubation	9
Average number of days required for incubation	13.20±.13
Coefficient of variation (incubation)	26.96±.75
Average daily mean temperature (based on hourly temperatures) for period for all eggs	74.5
Number of eggs hatched	339
Coefficient of correlation between mean temperature and length of incubation period	—.705±.018

RELATION OF TEMPERATURE TO THE LENGTH OF THE INCUBATION PERIOD.

A comparison of Table VII and Table VIII shows that the daily mean temperature during the incubation period was lower during 1915 than during 1916 and that the incubation period for 1916 averaged 2.6 days less than during the preceding year. In order to determine the relationship that exists between the daily mean temperature and the incubation stage of the cherry leaf-beetle, we have calculated the coefficient of correlation between these two phenomena using the data of 1916. This coefficient was found to be —.705 ± .018 and is rather high, showing there is marked correlation between the two phenomena. The coefficient is negative, which means that as the temperature increases the length of the egg stage decreases. From the regression equations we learn that on the average an increase of one degree in the daily mean temperature of the incubation period decreased the period by practically one day in our cages.

While the above analysis shows the important part temperature plays in determining the incubation period, it should also be noted that it is not the only factor to be considered. As has been observed with other species of insects, there existed marked variation in the length of the period of incubation in the same cage where the eggs were of the same age and presumably were subjected to identical conditions. Examples of this variation may be noted in lots 1, 5, 7 and 8 of Table VIII.

HATCHING.

Time of hatching of first, maximum number and last eggs.— The period during which hatching occurs extends from the latter part of June until the latter part of August of a normal year. The incubation of the eggs is influenced by the weather as noted above and during abnormal seasons variations occur in the length of the hatching period. It appears from the data that the maximum time during which hatching takes place is two months.

During 1915 the first larvæ were found in their native haunts at Fredonia on July 1, and Cushman[1] and Isley record larvæ hatching in their cages as late as August 18. These facts would give seven weeks as the period during which hatching occurred during this season.

During 1916 the first larvæ of the season were found both in the field and in breeding cages on June 23, but the eggs hatched very slowly until after July 4. The maximum number of larvæ emerged from July 12 to 31. With the eggs under confinement the hatching period ended about August 20, and during the months of July and August the weather was quite warm. The hatching period extended slightly over eight weeks, during the first two and last three weeks of which the relative number of larvæ emerging was rather small.

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[1] Loc. cit. p. 17.

THE LARVA.

ACTIVITY OF LARVA AFTER HATCHING AND NATURE OF FEEDING.

After emerging from the egg the larvæ ascend the tree at the base of which the eggs were laid and begin feeding on the foliage. If the tree happens to be a bird cherry the insects are able to develop to full growth, but if the eggs are placed at the base of another species of cherry the larvæ feed for a short time and if unable to find foliage of the bird cherry they have, according to our observations, invariably succumbed before passing thru the last instar, most of them dying during the first instar. The larvæ are active creatures, capable of crawling rapidly, and they feed almost entirely on the underside of the foliage. They eat the lower epidermis and the parenchyma, but generally do not eat thru the palisade layer and the upper epidermis. Occasional feeding occurs on the upper surface of the leaf. The nature of their feeding is shown in Plate XL. The injured portions of the leaves turn brown and portions of the palisade layer and the upper epidermis die and drop out, thus causing the foliage to have a ragged appearance.

FOOD PLANTS OF LARVA.

At the time of the outbreak of the cherry beetle during 1915 attempts were made to rear larvæ on foliage of the choke cherry, rum cherry and cultivated sour cherries and peaches, but in no instance was it possible to rear them beyond the second instar, and the majority of the creatures died during the first instar. These experiments were repeated in 1916 with identical results. Cushman[1] and Isley, on the basis of their studies during 1915, reported similar experiences. In the cages we were able to rear the larvæ only on the bird cherry, and in all field observations the only foliage on which the larvæ have been observed to reach maturity was that of this plant. It is for this reason that *P. pennsylvanica* has previously been designated as an *essential food plant* of this insect. It appears that infestation of cultivated cherries and peaches has its origin in the bird cherry. Observations indicate that where these fruits are grown outside the range of the bird cherry they are seldom attacked, and the probability of injury by the insects decreases directly as the distance from the range of the bird cherry increases. These facts also seem to warrant the statement that cherry and peach growers need pay no attention to any stage of the pest except the adult. The intermittent character of the outbreaks may be explained by the fact that only during periods of abnormal numbers of adults, especially if food be scarce, do these insects migrate to cultivated trees.

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[1] Loc. cit. p. 2.

GROWTH OF LARVA.

The larvæ feed voraciously and grow rapidly, especially during the warm periods. Two molts occur during the feeding period and a third molt takes place upon transformation to the pupal stage, which make three larval instars for this species.

Molting.— In molting the skin splits along the median line of the back, usually extending from the occiput down the thorax over several segments of the abdomen.

The larva first withdraws its head and thorax and after securing a hold on the leaf by means of its feet gradually withdraws the abdomen. During a portion of this process the larva was motionless and this quiescent period was followed by writhing motions to withdraw portions of the body from the skin. These operations were repeated at intervals of several minutes. The exact length of time required to molt has not been noted but on July 23 a larva was found in the course of molting at 3:20 P. M., at which time the head and body were pea green in color, the head being slightly lighter in shade. The spiracles were black and the legs were the same color as the head. At this time the larva had the shed skin on the abdomen from the eighth to the anal segment and it was motionless. The specimen was then closely observed and notes were made of any changes in its appearance as follows:

3:25 P. M. Head of the same color as at 3:20 P. M., body slightly darker, feet intermediate in color between that of head and body. A few writhing movements of the body, head raised several times at short intervals, then motionless again. Head measured .54 mm. in diameter which shows that the larva was in the second instar.

3:30 P. M. Color of head as before, body slightly darker, feet almost same color as body, first appearance of the formation of spots, larva generally quiet.

3:35 P. M. Head darker, body becoming an olive green. The larva was active at longer intervals than in preceding periods.

3:37 P. M. Larva leaves molted skin. Head a light olive green, body becoming quite dark, all the darker areas a dark olive green.

3:40 P. M. Head exhibiting same color as at 3:35 P. M. Traces of black begininng to show in the spots. Anal plate not as dark as the spots.

3:45 P. M. All portions of the body growing gradually darker. Spots prominent.

4:00 P. M. Head of a dark olive green with traces of black. Spots and anal plate very dark but not fully colored, legs slightly lighter than the body, claws and tarsi almost black. Larva resting quietly.

4:05 P. M. Body and legs almost black, head slightly lighter than the body. Larva crawling.

4:15 P. M. Body and legs black, head a dark brown.

These notes show that the act of molting may consume at least twenty minutes and that about an hour is required for the full development of the color of the larva.

Increase in the size of larva.— In order to measure the rate of growth, individual larvæ were placed in separate cages shortly after hatching and these were carefully observed until they entered the soil to pupate, daily measurements being made of the head diameter and length of each individual. Fifteen larvæ under observation passed thru the three instars and finally entered the soil. The measurements of these were made under a compound microscope with a lens arrangement whereby one space of the micrometer was equal to .0325 mm.

A leaf upon which the larvæ were resting was placed under the objective and the mirror set to reflect light thru the leaf, thus making it easier to note the position of the image on the micrometer stage. Owing to the size of the larvæ no measurement could be carried to a greater degree of refinement than the space of one division on the micrometer.

The diameter of the head of the larva of the cherry leaf-beetle, as is true of other species, does not increase during an instar, but the change in size occurs at the time of molting, while the epidermis is capable of expanding and before the chitin has become hardened. The average diameter of the head for the several instars was found to be as follows: First instar, .38 mm.; second instar, .54 mm.; third instar .76 mm. There was some variation in the sizes of the head in the different larvæ, as follows: First instar, from .36 to .39 mm.; second instar, .52–.57 mm., and third instar, .72–.78 mm.

The length of the body varies with the development of the larva, growth being constant between molts. There is no doubt considerable difference in the sizes of different individuals during each period of growth, but as it would have required a larger number of measurements to ascertain the extent of variation than the time at our disposal would allow, no effort was made to determine it. The variation in length of larvæ for each instar for a goodly number of specimens is as follows: First instar from 1.6 to 3.3 mm.; second instar, 2.3–4.5 mm., and third instar, 3.6–7.2 mm.

DURATION OF THE SEVERAL INSTARS AND THE LARVAL STAGE.

Observations of the length of the several instars were made on fifteen larvæ over a period ranging from July 13 to August 7. The data are given in Table IX.

TABLE IX.— STAGES OF DEVELOPMENT OF CHERRY LEAF-BEETLE FROM LARVA TO ADULT. FREDONIA, N. Y., 1916.

Lot.	Date of hatching.	Date of 1st molt.	Length of 1st instar.	Date of 2nd molt.	Length of 2nd instar.	Date of entrance into ground	Length of 3rd instar.	Total feeding period.	Date of emergence.	Time in ground.	Period from hatching to adult.
	July.	July.	Days.	July.	Days.	July.	Days.	Days.	Aug.	Days.	Days.
1	13	17	4	21	4	23	2	10	.....	.....	....
2	13	17	4	19	2	23	4	10	.....	.....	....
3	13	19	6	22	3	25	3	12	8	14	26
4	15	18	3	21	3	25	4	10	.....	.....	....
5	15	18	3	21	3	25	4	10	8	14	24
6	17	22	5	25	3	28	3	11	10	13	24
7	17	21	4	26	5	29	3	12	.....	.....	....
8	17	21	4	25	4	28	3	11	10	13	24
9	17	22	5	24	2	27	3	10	.....	.....	....
10	17	21	4	23	2	25	2	8	.....	.....	....
11	17	21	4	25	4	28	3	11	.....	.....	....
12	18	21	3	24	3	27	3	9	9	13	22
13	19	24	5	26	2	31	5	12	13	13	25
				Aug.		Aug.					
14	24	29	5	3	5	7	2	12	.....	.....	....
15	24	29	5	1	3	5	4	12	23	18	30

Average for different larval instars: 1st, 4.3 days; 2d, 3.2 days; 3d, 3.2 days. Period in soil 10.7 days.

As the temperature during the months of July and August of 1916 was considerably above normal, attention is called to the studies on the different larval instars by Herrick [1] and Matheson at Ithaca and by Cushman [2] and Isley at North East, Pa. The observations were made in 1915 during the months of July, August and September, which were cool and wet, with temperatures below normal. The data dealing with each instar are considered separately.

First instar.— In Table X there are summarized the more important facts by the foregoing observers, as well as our own, which bear on the duration of the first instar.

TABLE X.— LENGTH OF THE FIRST INSTAR OF THE LARVA OF THE CHERRY LEAF-BEETLE.

	Herrick & Matheson	Cushman & Isley	Author
Maximum length (days)...	7	11	6
Minimum length (days)...	4	4	3
Average length (days)....	5.9	4.8	4.3
Period of observation.....	July 23–30, 1915	Aug. 11–24, 1915	July 13–29, 1916

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[1] Loc. cit., p. 947.

[2] Loc. cit., pp. 10–17.

It will be noted that the longest period required for a larva to pass the first instar was eleven days and the shortest period three days. The average length of time for this stage as based on the records of two years in the Lake Erie valley is 4.6 days. It is unsafe to make a general average of the foregoing three sets of observations, owing to the fact that the climate of Ithaca is different from that of the Lake Erie valley, while the climate of North East, Pa., and Fredonia are quite alike, both being situated near Lake Erie and only thirty miles apart. As Ithaca is located in the plateau region of southwestern New York its climate resembles more closely that of the southern tier of counties than does that of Fredonia, wherefore the data from the former should be fairly representative of the native habitat of the insect.

Second instar.— The data dealing with this instar are summarized in Table XI.

Table XI.— Length of the Second Instar of the Larva of the Cherry Leaf-beetle.

	Herrick & Matheson	Cushman & Isley	Author
Maximum length (days)	5	7	5
Minimum length (days)	3	3	2
Average length (days)	4.3	3.6	3.2
Period of observation	July 27–Aug. 4, 1915	Aug. 5–29, 1915	July 17–Aug. 3, 1916

The longest period for the second instar was seven days, the shortest two days and the average for two years in the Lake Erie valley was 3.4 days.

Third instar.— The data on this instar are tabulated in Table XII.

Table XII.— Length of the Third Instar of the Larva of the Cherry Leaf-beetle.

	Herrick & Matheson	Cushman & Isley	Author
Maximum length (days)	6	8	5
Minimum length (days)	3	3	2
Average length (days)	5	4.1	3.2
Period of observation	Aug. 1–9, 1915	Aug. 10–Sept. 5, 1915	July 19–Aug. 7, 1916

The maximum length of the third instar is 8 days and the minimum 2 days, while the average period for two years at Fredonia and North East, Pa., is 3.7 days.

TOTAL FEEDING PERIOD OF LARVA.

The data bearing on this point consists of records by Herrick[1] and Matheson on nine larvæ, those of Cushman[2] and Isley on two hundred twenty-seven larvæ, and our observations on one hundred forty-four larvæ. These are tabulated in Table XIII.

TABLE XIII.— TOTAL FEEDING PERIOD OF LARVA OF THE CHERRY LEAF-BEETLE.

	Herrick & Matheson.	Cushman & Isley.	Author.
Maximum length for single larva (days)	17	20	24
Minimum length for single larva (days)	12	10	8
Average length for single larva (days)	15.2	12.3	12.3
Period of observation	July 23–Aug. 9, 1915	Aug. 5–Sept. 5, 1915	July 4–Aug. 13, 1916

From these figures it would appear that from twelve to thirteen days constitute the average period of the larvæ during a normal year in the Lake Erie valley. The Ithaca records indicate that on the Alleghany plateau of western New York the average length of life of the larva may be several days longer.

PUPATION.

When the larvæ have completed their growth they burrow into the leaf mold or, if not present, into the soil, which they penetrate a very short distance, usually about one centimeter, where they form a small spherical cell. Sometimes the larva forms scarcely any cell but transforms underneath rubbish. Moisture appears to be the chief factor in determining the depth of these cells. In such situations the larvæ pupate and the pupæ later transform to adults. The larvæ do not transform immediately on their entrance in the ground but require a number of days before undergoing a change in form. Cushman[1] and Isley found that the period was from five to eight days, depending on the temperature, and they also found the pupal stage varied from seven to eleven days. By means of Comstock root cages efforts were made to obtain pupæ, where they could be under constant observation, but our efforts resulted in failures. The only data obtained that bears upon the duration of the pupal period were the number of days that the insects were actually in the ground. In our cages there was considerable mortality, and of one hundred forty-four larvæ that entered the ground only fifty beetles finally emerged. The data bearing on the extent of the period occupied by the insect in the ground are summarized in Table XIV.

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[1] Loc. cit. p. 947.

[2] Loc. cit. pp. 12 and 14–17.

TABLE XIV.— PERIOD OF EXISTENCE OF INSECT IN THE GROUND DURING PUPATION.

	Herrick & Matheson.	Cushman & Isley.	Author.
Maximum period (days)	20	28	23
Minimum period (days)	17	14	12
Average period (days)	18.3	22.4	15
Number of individuals	9	563	50
Dates	Aug. 5–28, 1915	Aug. 5–Sept. 18, 1915	July 18–Sept. 2, 1916

As will be observed, there is quite a little variation in the figures given by the different observers. This is believed to be due to variations in climate but may be partially due to differences in methods of rearing the insects. As will be explained later, weather conditions during 1915 and 1916 were somewhat abnormal, the two years differing greatly in the Lake Erie valley. The climate of Ithaca during 1915 differed markedly from that of the Lake Erie region.

PERIOD FROM HATCHING TO EMERGENCE OF ADULT.

The total period of development of the cherry leaf-beetle from the larval stage to adult was found by all observers to vary considerably, and in our studies there was a difference of twenty days between the minimum and maximum periods. As shown in Table XV the records indicate that about one month is required for the average individual to reach the adult stage after hatching from the egg. If to these figures there are added thirteen days for the egg stage, the period for the complete development of the insect ranges from forty to forty-seven days for the Grape Belt along the shores of Lake Erie and about forty-eight days on the higher lands to the south.

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[1] Loc. cit. p. 12.

TABLE XV.— TOTAL DEVELOPMENTAL PERIOD OF THE CHERRY LEAF-BEETLE.

	Herrick & Matheson.	Cushman & Isley.	Author.
Maximum length for a single individual (days)	36	40	40
Minimum length for a single individual (days)	32	31	22
Average length for a single individual (days)	34.6	33.8	27.2
Period of observation	July 23–Aug. 28, 1915	Aug. 5–Sept. 18, 1915	July 4–Sept. 2, 1916

CLIMATE VARIATION DURING 1915 AND 1916.

The climatic conditions during the developmental period of the cherry leaf-beetle during 1916 are shown in Figs. 18 and 19.

In order that the results of the studies by various workers may be fairly compared the meteorological conditions that prevailed in the different localities where the insect was studied should be noted (Table XVI). In regard to 1915 we have used the weather reports of Westfield, N. Y.,[1] which is only fourteen miles from North East, Pa.; and the location of the cooperative observer is about the same distance from Lake Erie and at only a slightly greater elevation than the laboratory where Cushman and Isley conducted their studies. We have also used the Westfield records to secure the departure from normal for 1916 since the Fredonia records do not cover a long enough period for such a calculation. As the Westfield observer is only fourteen miles distant and only slightly higher than the Vineyard Laboratory no great error is introduced. The amount

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[1] Wilson, W. M. Climatological Data, New York Section. U. S. Dept. Agr., Weather Bureau. June to September 1915; and June to August 1916.

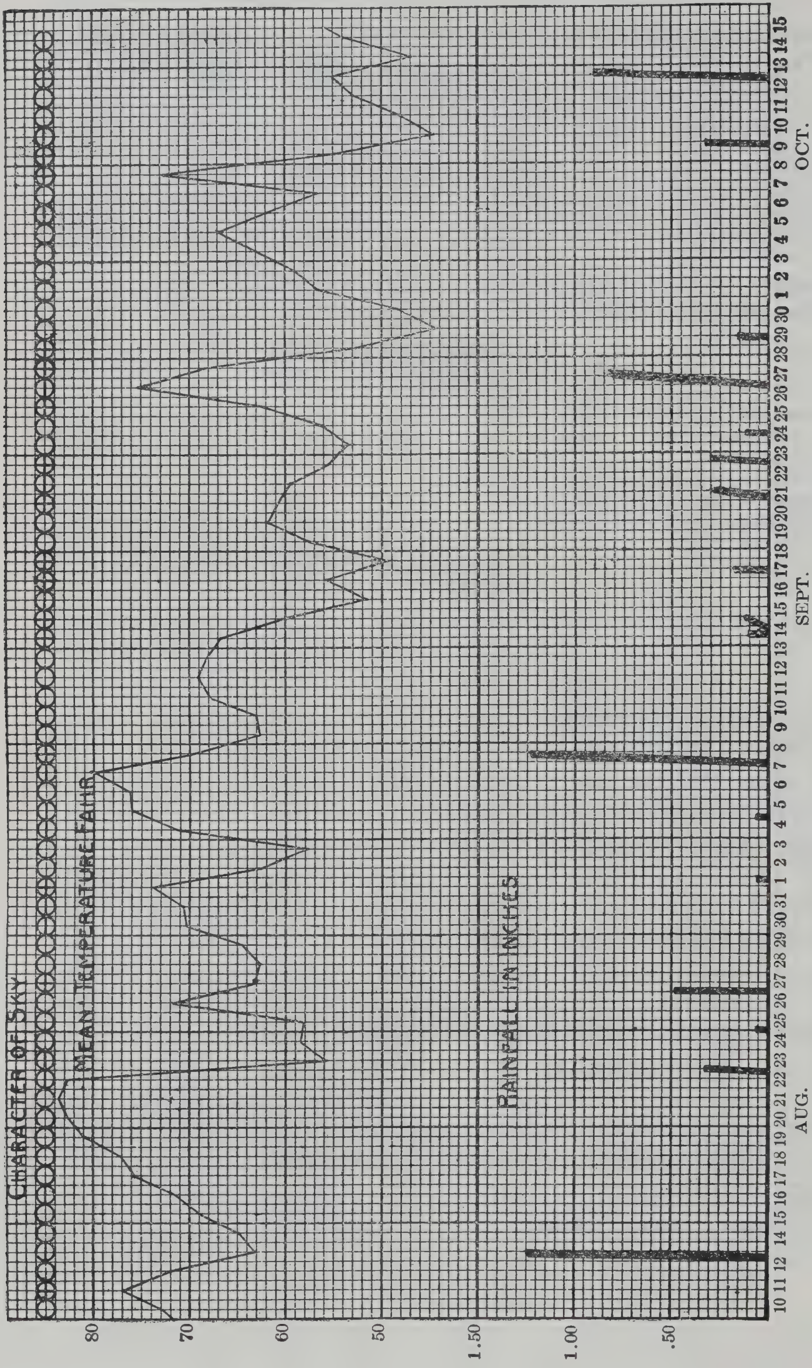

FIG. 19.—CHART SHOWING CLIMATIC CONDITIONS FROM AUGUST 10 TO OCTOBER 15, 1916, AT FREDONIA, N. Y.

of cloudiness during both years except at Ithaca [1] and all other records for 1916 were obtained from the report of our laboratory at Fredonia.

TABLE XVI.— CLIMATIC CONDITIONS DURING 1915 AND 1916 AT WESTFIELD, ITHACA AND FREDONIA, N. Y.

	Westfield 1915.	Ithaca 1915.	Westfield 1916.
Temperature, departure of mean from normal.	June —1.5*	....	—3.9
	July —3.3	—1.4	+3.3
	Aug. —3.1	—2.0	+2.1
	Sept. +1.4*	....	....
Precipitation, departure of mean from normal.	June —0.70 in.	....	—0.39 in.
	July +7.42 in.	+2.43 in.	—0.97 in.
	Aug. +2.02 in.	+0.46 in.	—0.72 in.
	Sept. +0.14 in.	....	....
	Fredonia 1915.		Fredonia 1916.
Percentage of clear days during period in which observations were made on developmental stages............	34	11	66
Percentage of cloudy days (same period).........................	34	38	17
Percentage of partly cloudy days (same period)....................	32	51	17

* From the records of the observer at Volusia (5 miles from Westfield but 500 feet higher) since the data are not given in the Westfield records.

SUMMARY OF THE SEASONAL HISTORY.

With respect to western New York the insect emerges from the pupal cell as an adult during August and the early part of September. In 1916 the adults emerged from July 31 to September 2, but during 1915 adults emerged as late as September 18. Little feeding is done at this season and during the latter part of September the beetles seek sheltered locations in which to pass the winter. They burrow for a short distance into the soil and form their hibernating cells, where they remain for nearly eight months. Emergence from hibernation takes place during the latter part of May of a normal year. After the beetles abandon their winter quarters they feed voraciously for a time on the bird cherry and then begin to disperse. This movement of the beetles is usually to other bird cherry trees. However,

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[12] Furnished thru the kindness of Prof. W. M. Wilson.

when vast numbers of the beetles have escaped the rigors of winter, especially if the preferred food is scarce, the insects migrate to cultivated cherry and peach trees. The migratory period usually occurs during the early part of June, altho some dissemination may occur during the last week of May. In 1915 the period extended from about June 1 to June 12, and during 1916 the migration occurred during the first and second weeks of June.

Mating occurs shortly after the beetles first feed in the spring and continues during the entire month of June and a portion of July, continuing about six weeks.

During 1916 the earliest eggs were laid June 5 and the last eggs were laid in breeding cages August 9. The total egg-laying period was sixty-six days. The greatest oviposition activity occurred during the last week of June and the first week of July. The average egg-laying period for a single female in the breeding cages was twenty-eight days. The greatest number of eggs laid by a single female was 294, the least number was 10, and the average number was 93. During 1915 the incubation period averaged 15.8 days, with a maximum of 21 days. The incubation period during 1916 averaged 13.2 days with a maximum of 23 days. Hatching occurred from June 23 to about August 20, 1916, or over a period of practically two months.

The larvæ required an average of about 12 days from hatching until they entered the soil. The longest period was 24 days, and the shortest 8 days. The larvæ upon reaching full growth entered the soil and formed cells in which they pupated. The period spent in the ground varied in 1916 from 12 to 23 days with an average of 15 days. In 1915 Cushman [1] and Isley found the period spent in the ground varied from 14 to 28 days with an average of 22.4 days. These variations are believed to be due to differences in temperature, and 19 days appears about the average for a normal season. The total developmental period of the cherry leaf-beetle varied from 22 to 42 days with an average of 27 days during 1916. Cushman [2] and Isley in 1915 found this period to average about 34 days. It is concluded that one month is about the average length for the developmental period during a normal season. The time from the deposition of the eggs until the emergence of the adult averages about 43 days.

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[1] Loc. cit. pp. 12, and 14-17.

[2] Loc. cit. pp. 13-17.

A graphic summary of the seasonal history of the insect is shown in Fig. 20.

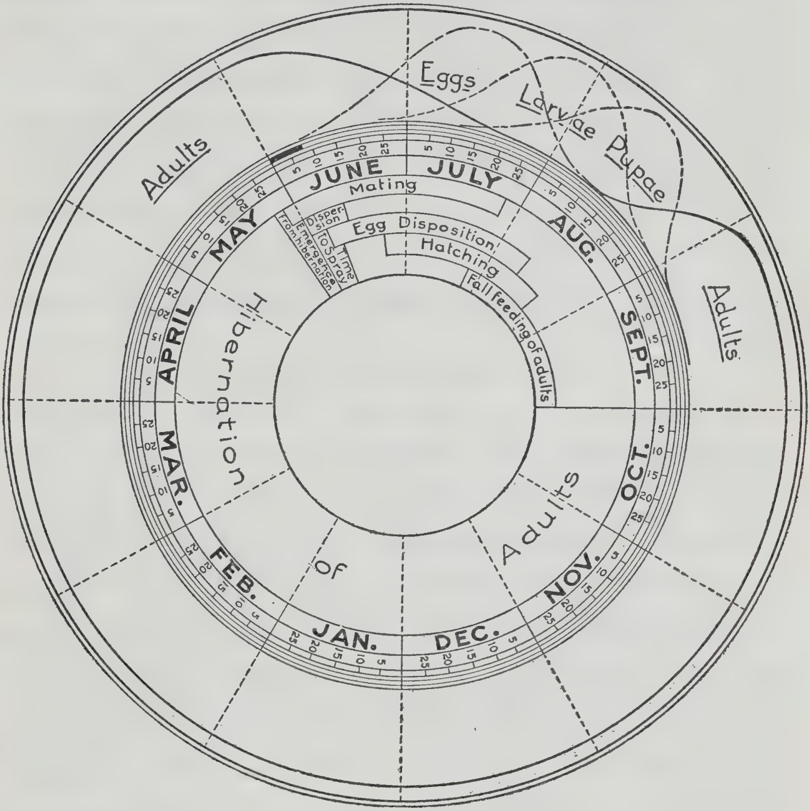

Fig. 20.—Diagram Showing the Seasonal History of the Cherry Leaf-beetle as Observed at Fredonia, N. Y., During 1916.

NATURAL DISSEMINATION.

DISPERSION DURING THE LARVAL STAGE.

The first movements of the larvæ are to obtain food and are chiefly vertical owing to the eggs being laid at the bases of trees. When branches intertwine there doubtless is some movement horizontally, but this activity apparently carries the insect only short distances.

SUMMER AND FALL FLIGHTS OF THE BEETLES.

After the emergence of the beetles during August and early September it is possible that some movements by the insects occur. As the hibernating places of these insects are usually at the bases of the trees upon which they feed and as the food supply at this season is usually abundant there is little reason to believe that the beetles attempt any extensive flights. Observations made in the habitats of these beetles during the late summer have shown the adults to be rather sluggish and not inclined to fly as readily as in the spring. This behavior on their part is not believed to be due to temperature. At this season they are not voracious feeders, even when surrounded with an abundance of food. In cages they fed so sparingly as to lead one to wonder how they were able to maintain life. In feeding they seemed to prefer foliage that was near the ground.

SPRING DISPERSION OF BEETLES.

All movements of the species are insignificant in comparison with the dispersion of the beetles in the spring. During 1915 and 1916 this began slowly during the latter part of May and early June, suddenly reaching a maximum at the end of the first week of June and gradually diminishing until the middle of this month when dispersion was practically concluded. During 1917 the beetles were very scarce and no migration was observed. Observations during 1916 indicated that a few eggs were deposited before this movement occurred, but the bulk of the eggs were laid after the migration. Several conditions may precipitate and intensify this movement, of which the principal factors are apparently the existence of enormous numbers of beetles and scarcity of food. Restriction in the means of subsistence may be due to various causes but the destruction of the foliage of the bird cherry by other insects or by freezing have apparently been chiefly responsible for the migration to incidental food plants during the three years that the insect has been under observation.

The beetles fly with the wind and the direction and velocity of the wind largely determine the course and extent of their movement into new territory. No data are available showing how far the beetles may travel in a single flight, but observations indicate that it is only a short distance and they then alight on any plant that is at hand. If the selection does not prove to be to their liking they resume their flight. The reports of fishermen finding the beetles on stationary floats several miles off shore in Lake Erie would indicate that the insects flew this distance. It is claimed that they were found on driftwood ten miles from shore in this lake, but in such instances we were unable to determine the distance the object drifted after the beetles alighted. On land the combined flight of the beetles was extensive. Since most of the beetles came from the northern part of Pennsylvania and southern New York and they were found flying several miles out over Lake Erie there are good reasons for believing that during 1915 vast numbers of the insects flew about forty miles in Chautauqua County.

It is presumed that the beetles are able to locate cherry trees, but it should be noted that during the invasion of 1915 the beetles were observed alighting within one hundred feet of these trees on all manner of plants upon which they did not feed, and that afterwards they sought cherry trees.

RELATION OF WATER TO DISSEMINATION.

Small bodies of water do not appear to influence the spread of the insect, but large bodies of water, such as the Great Lakes, are barriers to its progress. It is possible that beetles which alight on driftwood may be washed to a distant shore, but from the behavior of the insects during 1915 apparently not many are carried by this means. However, the beetles would quite easily cross a territory of this width if no water intervened. While the beetles are not easily drowned, nevertheless many are killed in the water and it is difficult to imagine under what circumstances large bodies of water would assist in the dispersal of the species.

NATURAL CONTROL.

Under this heading there are considered drowning of adults, deposition of eggs in situations which are unfavorable to development of larvæ, and parasitic and predaceous enemies.

DROWNING OF ADULTS.

During 1915 it was observed that numbers of these insects were drowned in Lake Erie. The beetles, conveyed either thru their own powers of flight or assisted by heavy winds, were found in great numbers in the lake at considerable distances from shore. Some of these alighted on driftwood and some fell in the water not far from land and were washed ashore alive, but the vast majority fell into the water and were drowned, their dead bodies being later washed up by the waves until they formed windrows on the lake beaches.

Counts of the insects along the shore at Westfield showed an average of fifty dead beetles to each linear foot of beach. If the destruction of the species was uniformly as great along the entire forty miles of lake shore in Chautauqua County, as there are reasons for believing, the total fatality according to these estimates was not less than 10,500,000,000 beetles.

EGG SITES UNFAVORABLE TO LARVAL DEVELOPMENT.

As previously stated, the larvæ of the cherry leaf beetle did not mature on any of the orchard fruits, so that beetles which deposited their eggs only on cultivated cherries and peaches died without maturing progeny. In the invasion of a region where the bird cherry exists only in small numbers there is perhaps no other factor which makes for the more rapid decline of the insect in importance than the inability of the larvæ to subsist on any other plant than its native host.

REFORESTATION.

Cutover land which has grown up to bird cherry and other less valuable vegetation, when protected from fire and grazing, becomes replanted with the more valuable deciduous and coniferous trees which crowd out the bird cherry and other forest margin species of plants. Where this reforestation is extensive it naturally controls the cherry leaf-beetle by decreasing its food plants. Such an effect would be marked in the uplands of the Alleghany plateau if better fire protection were maintained and more systematic tree planting practised.

PREDATORY INSECT ENEMIES.

Only one insect enemy of the cherry leaf-beetle has been recorded. This is a carabid beetle, *Lebia ornata* Say, which has been reported by Cushman[1] and Isley as attacking and killing the adult beetles soon after they emerged from the soil.

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[1] Loc. cit. p. 19.

BIRDS.

During the invasion of the beetles in Chautauqua County, Mrs. H. M. Putnam of Fredonia, who is a student of bird life, observed a pair of cedar waxwings (*Bombycilla cedrorum* Vieill.) feeding extensively upon the insects. The pair returned several times to a sour cherry tree and during each visit they captured considerable numbers of the adults. With a pair of field glasses she was able to count over fifty beetles that were eaten by the birds. Notwithstanding the great abundance of the species, it is a singular fact that none of those who have carefully studied the cherry leaf-beetle ever had their attention directed to birds as important predatory enemies of this insect.

ARTIFICIAL CONTROL.

Since the larvæ do not feed on the foliage of cultivated fruits and the adults only are destructive, the experiments to devise efficient control measures were directed largely against the beetles. Special emphasis was placed on arsenicals and nicotine solutions in these operations and the principal details of the different tests are as follows:

TEST NO. 1 WITH ARSENICALS.

The first experiments during 1915 for the control of the beetles were made on June 7 when they were first discovered at Fredonia. The tests were made on young cherry and peach trees which at that time were partially defoliated. The cherry trees were two years old from the time of planting and for the purpose of experiment were divided into two plats. Plat 1 was treated with a mixture of 2 pounds of dry arsenate of lead in 50 gallons of water, while Plat 2 was sprayed with a mixture of 4 pounds of paste arsenate of lime in 50 gallons of water. The trees were thoroly sprayed so as to wet all surfaces of the leaves. In addition a block of peach trees received an application of arsenate of lead as above for cherries.

A light rain occurred during the night. As many beetles continued to feed on the trees and the owner feared that the rain had washed off the poison, the plats were, without the author's knowledge, resprayed as before. An examination of the trees on July 9 showed that there were many dead beetles on the ground and that the work of defoliation had been effectively stopped. The cherry trees sprayed with arsenate of lime showed less infestation on July 9 than did the plat sprayed with arsenate of lead. In no instance was there any evidence of injury to the cherry foliage by arsenate of lime nor to peach foliage by arsenate of lead. It may be added that in none of the experiments during 1915 by the author was there any damage to peach foliage either by arsenate of lead or arsenate of lime, altho in several instances injury was reported by growers who used arsenate of lead.

TEST NO. 2 WITH AN ARSENICAL AND NICOTINE SOLUTION.

A rather extensive series of experiments for the control of the adults of the cherry leaf-beetle were made June 8–10, 1915, in the cherry and peach orchards of R. J. Paschke near Fredonia.

On June 8 three plats of trees that were fairly uniform in size were treated respectively as follows: Plat 1 received an application of $\frac{1}{2}$ pint of nicotine sulphate (40 per ct. nicotine) in 80 gallons of water; Plat 2 was sprayed with bordeaux mixture after the formula of 4–4–50, 3 pounds of paste arsenate of lead and 5 ounces nicotine sulphate. Plat 3 was sprayed with a mixture of 3 pounds of paste arsenate of lead, 2 quarts molasses and 50 gallons of water. All trees were thoroly sprayed in order to wet the upper and undersides of the foliage. Whenever nicotine solution was applied the beetles which dropped from the trees at the beginning of the treatment were afterwards thoroly sprayed while on the ground. This operation, it may be added, required the employment of larger quantities of spraying mixture but was effective.

An examination on June 9 of the trees in plats 1 and 2 showed many dead beetles on the ground with a corresponding decrease in the number of the insects on the trees. The trees in plat 3 were in a much less satisfactory condition as many beetles were feeding on the leaves and only a few had succumbed to the treatment. The trees sprayed with bordeaux mixture, arsenate of lead and nicotine solution presented markedly superior conditions as compared with the remaining plats. Altho the number of beetles killed was no larger, perhaps, than on the trees sprayed with nicotine solution alone, yet the number of insects feeding on the foliage was less than on the trees not receiving the combined treatment. The foliage sprayed with bordeaux mixture and arsenate of lead was thickly coated with the spray which appears, in addition to its toxic properties, to have acted as a repellent to the beetles. It should, however, be added that the trees showed more infestation on June 10 than on June 9, due to the constant migration of beetles. Notwithstanding the re-infestation, the trees in each plat were, as a result of a single spraying, saved from defoliation altho considerable feeding occurred on the trees sprayed with molasses and arsenate of lead.

TEST NO. 3 WITH ARSENICALS.

On June 10 large Montmorency trees were thoroly sprayed with bordeaux mixture after the formula of 4–4–50, to which were added 3 pounds of paste arsenate of lime. Examination of the trees on June 11 showed excellent protection of the foliage, altho there was little evidence of insects having succumbed to the treatment. The freedom of the trees from the work of the insects appeared to have been due to the repellent properties of the mixture.

It is also important to note in this connection that the season of 1915 was very favorable for brown rot on cherries, owing to excessive rainfall and high humidity, but fruit on all trees sprayed with bordeaux mixture was harvested for the most part in excellent condition while with untreated trees there was considerable rot.

TEST NO. 4 WITH ARSENICALS.

During the afternoon of June 10 a number of young peach trees were sprayed with bordeaux mixture after the formula of 4–4-50 and 3 pounds of paste arsenate of lead, while other trees were sprayed with bordeaux mixture with 3 pounds of paste arsenate of lime. The beetles were killed by either treatment and the foliage for the most part showed little evidence of further feeding by the insects. Spraying of peaches with bordeaux mixture is considered an unsafe practice and it should be noted that in these tests there was no evidence of leaf injury or of defoliation of the trees by either of the treatments.

TEST NO. 5 WITH VARIOUS INSECTICIDES.

A block of young cherry trees of the variety Montmorency belonging to Mr. D. B. Belden in Fredonia became severely infested with the leaf-beetle, and on June 10 this planting was divided into plats which were sprayed as follows: Plat 1, Pyrox, one pound to eight gallons of water; Plat 2, Insectine, one pint to fifty gallons of water; Plat 3, nicotine sulphate (40 per ct. nicotine) one pint to eighty gallons of water; and Plat 5, nicotine sulphate, one pint to sixty gallons of water. All applications were very thoro, both surfaces of the leaves being drenched with the sprays. In applying the nicotine mixtures and Insectine special efforts were made to wet the insects with the spray. The tendency of the beetles to drop from the leaves before being wetted with the mixture showed the necessity of spraying them again while on the ground in order to allow none of them to escape. Insectine is primarily a poison, but it is, however, a combination of various insecticides and fungicides, containing some chemical substances that are toxic to insects by contact. It was to test the preparation as a poison and as a contact spray that the precaution of spraying the foliage as well as the beetles on the ground was observed.

All of the applications of the nicotine solution at different strengths killed many of the beetles; and from the numbers of dead insects on the ground it appears that thoroness of treatment was of more importance than the dilution of the spraying mixture. A serious limitation of nicotine sprays is plainly indicated in this experiment — they do not possess lasting toxic or repellent properties, so that trees treated with them are liable to be reinfested.

Many dead beetles were observed beneath the trees sprayed with Pyrox, and the numbers of the insects on the foliage was very small. The material appeared to exert a repellent effect in addition to its usual toxic action.

The trees sprayed with Insectine showed no dead beetles and the number on the foliage did not seem to be less than on untreated trees.

TEST NO. 6 WITH TREE TANGLEFOOT.

The discovery that the beetles lay their eggs at the bases of the trees and the belief that the larvæ would feed on the foliage led to the opinion that the adults might possibly be captured by adhesives.

It seemed advisable to undertake experiments to prevent oviposition by the beetles and any movement by the larvæ.

On June 18 several large peach and cherry trees were treated with Tree Tanglefoot. This was applied in a band about five inches wide, encircling the base, and was put either on the bark or on a band of muslin. The ground about the trees had been removed previously to eliminate any eggs that may have been present. The trees were mounded in such a manner that the ground covered about half of the band of Tanglefoot, i. e., the earth extended one-half of the distance to the top of the band. Other trees were mounded after having all eggs removed but the Tanglefoot was omitted.

An examination of these trees, June 21, showed that many beetles had been captured by the Tanglefoot and that at this date no eggs had been laid at the bases of the treated trees. In order to have more detailed data on the effect of the banding, the ground about two treated and two untreated trees was carefully examined on June 26 when counts were made of the eggs. The results of these counts are given in the following table:

Table XVII.— Number of Eggs of the Cherry Leaf-Beetle on Trees Treated with Tree Tanglefoot.

Untreated peach tree	224 eggs
Untreated cherry tree	227 eggs
Peach tree with Tanglefoot	25 eggs
Cherry tree with Tanglefoot	27 eggs

A general examination of the other treated and untreated trees indicated that similar conditions prevailed, and while these results are of interest in demonstrating what might possibly be accomplished by banding bird cherry trees in parks or arboretums, this method of protecting orchards from this insect is unnecessary since few if any of the larvæ are able to exist on the foliage of cultivated trees.

SUMMARY OF EXPERIMENTS.

Arsenate of lead at the rate of 4 pounds to 50 gallons of water or bordeaux mixture killed many of the beetles if care was exercised to cover the undersides of the foliage. The most lasting effect was secured by the use of arsenate of lead and bordeaux mixture.

Arsenate of lead and molasses in combination were not as effective nor did they possess the lasting properties of arsenate of lead with bordeaux mixture.

Arsenate of lime at the rate of 4 pounds to 50 gallons of water was apparently somewhat more efficient than arsenate of lead at similar proportions and there was no injury to the foliage of either cherry or peach. In combination with bordeaux mixture the arsenicals were equally effective.

Nicotine sulphate (40 per ct. nicotine) was found to be very effective when used as a contact insecticide at the rate of one pint with either 60 or 80 gallons of water or bordeaux mixture. One experiment indicated that even greater dilutions might be used but the increased care needed to thoroly wet the insects tended to offset the economy in the use of the stock material. The best results with nicotine extracts, so far as permanent effects are concerned, were secured by combining them with arsenate of lead and bordeaux mixture.

Pyrox used at the rate of one pound to eight gallons of water killed the beetles and also served as a repellent.

Insectine did not control the cherry leaf-beetle.

Tanglefoot reduced the number of eggs deposited at the bases of peach and cherry trees.

RESULTS OF TESTS BY OTHER EXPERIMENTERS.

Experiments by Cushman[1] and Isley to develop efficient spraying methods were largely directed to testing arsenate of lead and nicotine sulphate, and the results of their operations were similar to those described except that somewhat better results were obtained with arsenate of lead in combination with molasses. They also tested crude carbolic acid emulsion, using the formula of 10 pounds fish-oil soap, ¾ pint of crude carbolic acid and 50 gallons of water. This proved efficient as a contact spray but, as was the experience with nicotine sulphate, it lacked lasting toxic and repellent qualities.

CONCLUSIONS AND DIRECTIONS FOR CONTROL MEASURES.

In the light of the results of the foregoing experiments it appears that the cherry leaf-beetle can best be controlled on large cherry trees by spraying during the first week of June or as the beetles first appear, with bordeaux mixture (4-4-50) combined with either four pounds of paste arsenate of lead or two pounds of dry arsenate of lead. It is believed that paste arsenate of lime can be used with the bordeaux mixture in place of the arsenate of lead, but more experimentation is needed before it can be generally recommended. In spraying, the foliage should be thoroly covered on both the upper and lower surfaces. In addition to protection from several species of insects, another benefit from this treatment is the control of various diseases of fruit and foliage, which during some seasons are the cause of considerable apprehension among cherry growers.

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[1] Loc. cit. pp. 19–24.

For young cherry trees, especially those that have been set not more than two years, jarring the beetles into sheets placed under the trees, followed by the destruction of the beetles, is a satisfactory method of control. This is especially recommended when infestation is severe since the small amount of foliage present on young trees makes it possible for the beetles to defoliate them before the insects succumb to the poison if sole reliance is placed on an arsenical. After the number of beetles has been reduced by jarring, an application of bordeaux mixture with arsenate of lead should render the trees immune to further attacks.

The protection of peach trees is a more difficult problem because of the great danger of injury to foliage from applications of bordeaux mixture and arsenate of lead. If large trees are attacked by numbers of the beetles the only recourse appears to be an application of arsenate of lead at the rate of 4 pounds to 50 gallons of water. To every barrel of the spray add two pounds of lump lime to neutralize any soluble arsenic present in the mixture. Inferior brands of arsenate of lead should be avoided. With young peach trees, jarring the beetles into sheets spread on the ground will greatly reduce the numbers of the insects, and if the insects tend to increase in numbers again, follow jarring with an application of arsenate of lead. Bordeaux mixture should not be used on peach foliage.

Nicotine sulphate (40 per ct. nicotine) may be employed effectively against the cherry leaf-beetle if trees are first thoroly sprayed and then treatment is directed to the beetles which drop to the ground. In the experiments with tobacco mixtures the best results were obtained when one pint of the nicotine solution was added to 60 gallons of water, altho a dilution of one pint to eighty gallons of water proved very effective. The advantage of using nicotine solution is that it can be used on peach trees without injury to foliage. However, it possesses several disadvantages, which are the cost of application, owing to the rather excessive amount used per tree, and the necessity of frequent applications during the dispersion period of the beetles. Failures on the part of the growers to combat the pest satisfactorily with nicotine solution were largely due to lack of thoroness in spraying and neglect to spray the insects after they had dropped to the ground.

The use of a soap-carbolic acid solution as described above is to be recommended with the caution that, while it has generally proven safe to foliage, injuries to leaves of cherry trees have been reported.

Experiments with a commercial coating composition known as Tree Tanglefoot for capturing the beetles before they lay eggs at the bases of trees indicates that this method might be of use in controlling the larvæ of the cherry leaf-beetle on bird cherry in parks or arboretums.

While a cooperative effort to clear much of the waste land of bird cherry about farms might at first seem advisable, further study has shown us that during ordinary years these do not harbor the beetles, presumably because the proper conditions for hibernation do not develop; and only during years of severe outbreaks do the trees become infested. It therefore appears to be a good practice to allow these trees to remain that their fruit may serve as food for useful birds.

All efforts in reforestation that succeed in re-establishing new timber tracts on the waste, cutover and abandoned land of the Alleghany plateau region of western New York and Pennsylvania will not only help in increasing a state and national asset, but incidentally they will reduce the number of bird cherry trees and bushes, many of which are too young for producing fruit. It is the seedlings that spring up in large numbers after every fire that furnish food for the hordes of cherry beetles which overrun cherry orchards during years when the normal supply of food is restricted.

ACKNOWLEDGMENTS.

In our efforts to determine the distribution of the cherry leaf-beetle, we have corresponded with a number of entomologists and horticulturists who have had an opportunity to observe the species, or who have specimens in their private collections. The assistance rendered by them has been previously noted in this bulletin.

For extended notes on *G. cavicollis* and references to literature we are under obligations to

Prof. R. H. Pettit, East Lansing, Mich.;
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W. T. Davis, New Brighton, N. Y.;
C. W. Leng, New York City; and
H. E. Backus, North East, Pa.

The author is also indebted to W. F. Wight, Bureau of Plant Industry, Washington, D. C., and Prof. W. W. Robbins, Fort Collins, Col., for information relative to the exact boundaries of the range of *P. pennsylvanica.*

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THE ROSE LEAF-HOPPER.*

(*Typhlocyba rosæ* L.)

F. H. LATHROP.

The ornamental value of rose plantings is frequently distinctly curtailed if not actually destroyed through the activities of the rose leaf-hopper. This has long been known as an important rose pest, and occurs almost wherever roses are grown in Europe as well as in America. The presence of the insect to an injurious extent is plainly indicated by the whitening of the foliage, and easily confirmed by finding the nymphs and their cast skins on the underside of the leaves.

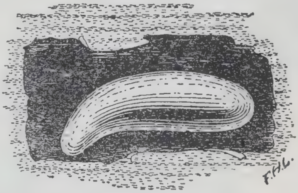

Fig. 21.—Egg of Rose Leaf Hopper.
(Enlarged.)

LIFE HISTORY.

In the late fall the eggs (Fig. 21) are deposited just beneath the bark of the mature stems, and it is in this stage that the insect passes the winter. About the middle of May, after the leaves have become well developed, the eggs hatch and the tiny, white or pale yellowish nymphs emerge (Fig. 22). These lively little creatures at once migrate to the underside of the leaves where they may be found feeding, but where they are not likely to be observed unless special search is made for them. The nymphs continue to grow until the latter part of May when the skin is shed for the fifth and last time, and the adult insects appear (Fig. 23).

The adult is a rather slender, winged insect about three-sixteenths of an inch in length, and, like the nymph, is white or of a pale yellow color. The insect now becomes exceedingly active, dodging around the stems as one approaches, and flying swiftly away when the plants are disturbed.

There are at least two, and possibly three, broods produced during the course of the summer. The adults of the first brood, leaving the rose to a large extent, migrate to numerous other food plants where most of the nymphs of the later broods are produced.

NATURE OF INJURY.

By far the most serious injury to the rose is due to the disfiguring of the foliage as a result of feeding. The insects in both the adult and the immature stages feed by puncturing the tissues and sucking away the juices from the leaves (Plate XLVII). Each feeding puncture produces a minute white spot which is especially apparent on the upper surface of the leaf. The affected leaves soon become thickly stippled with these white spots, and when the infestation is heavy, the spots run together so that the entire surface of the leaf becomes pale (Plate XLVIII), and the plant as a whole presents a displeasing, sickly appearance. Plants which have the leaves severely injured in this way are unable to make normal growth.

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* Reprint of Circular No. 55, May 10, 1917

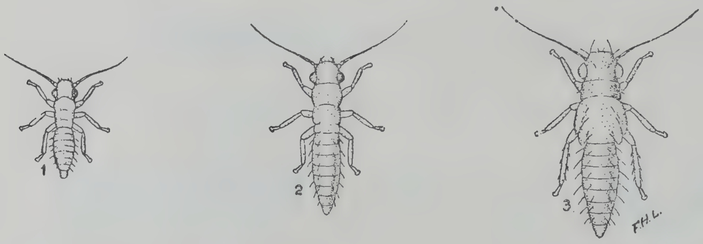

FIG. 22.— NYMPHS OF FIRST, SECOND AND THIRD INSTARS.
(Enlarged)

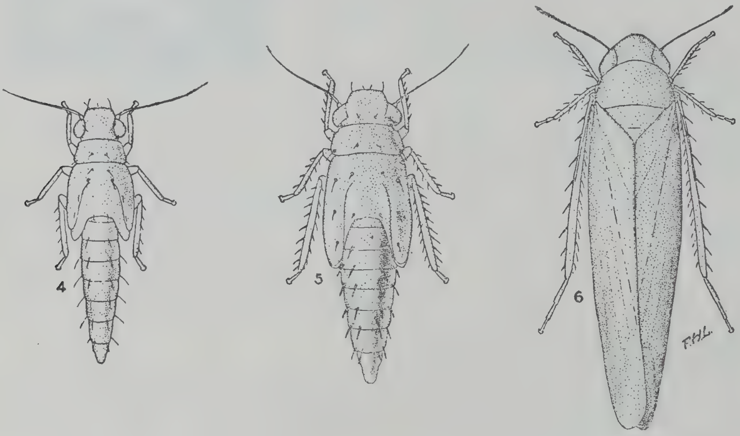

FIG. 23.—NYMPHS OF FOURTH AND FIFTH INSTARS, AND ADULT.
(Enlarged.)

VARIETIES AFFECTED

While the crimson rambler seems to be especially susceptible to attack, many varieties of roses have been found to sustain severe injury, and no variety appears to be immune. Besides the rose, many other plants, including apple, cherry, and many small fruits are subject to injury from this pest.

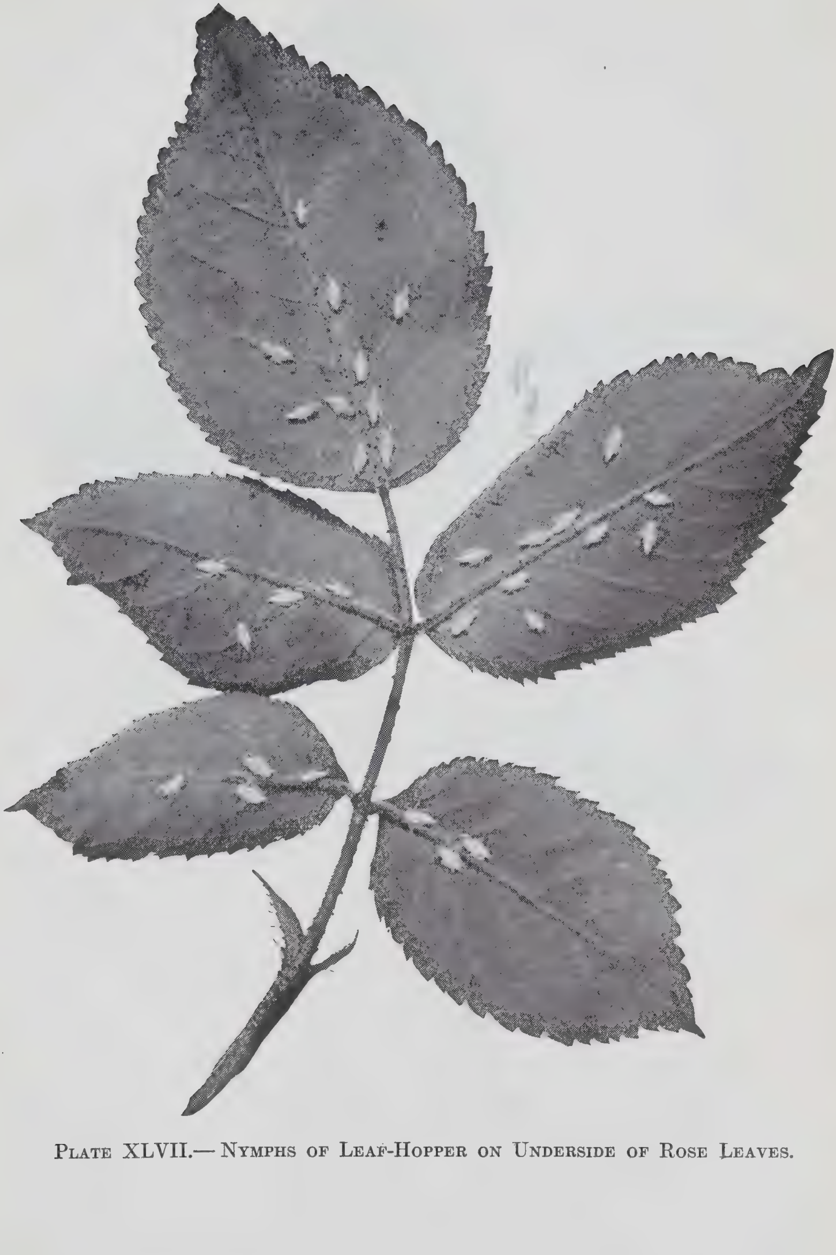

Plate XLVII.— Nymphs of Leaf-Hopper on Underside of Rose Leaves.

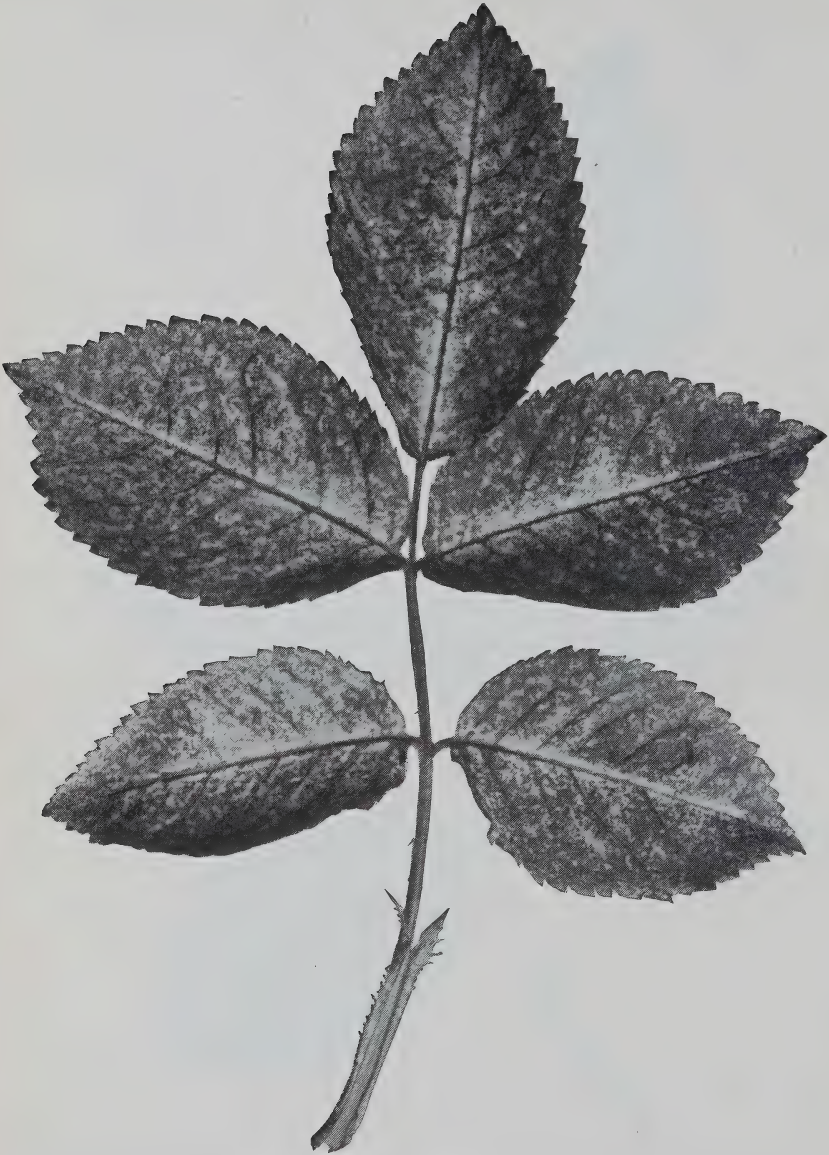

Plate XLVIII.— Rose Leaves Injured by Feeding of Leaf-Hopper.

NATURAL ENEMIES.

The rose leaf-hopper, as is fortunately the case with most of our insect pests, is attacked by predaceous and parasitic enemies which under normal conditions greatly reduce its numbers. The adults may frequently be seen entangled in spider webs, while the nymphs as well as the adults fall prey to the small jumping spiders which inhabit the plants upon which the hoppers feed. Birds must also be a factor in this connection, in spite of the fact that these tiny, active insects do not seem to be very attractive food to many birds. Without doubt the most efficient of these destroyers to be observed in this locality is a minute parasitic insect that matures within the leaf-hopper egg, which consequently fails to develop.

TREATMENT.

Although parasites render valuable service in the control of this pest, it frequently becomes necessary to supplement their work with artificial control measures. If treated at the proper time, this leaf-hopper is not difficult to hold in check. Affected roses should be thoroly sprayed while the insects are in the nymphal stages, using a solution of nicotine and soap in the proportions of three-fourths pint of nicotine sulphate and five pounds of soap to one hundred gallons of water. Where only a few plants are to be treated, the mixture may be prepared at the rate of one teaspoon level full (four cubic centimeters) of nicotine sulphate, 40 per ct., and two ounces of soap to one gallon of water. The spray is effective only while the insect is in the immature stages, for the adults fly so quickly that they cannot be reached with the spray. It will be seen from the life history that the treatment may be effectively made from the time of hatching until the last of May in this locality. It is advisable, however, to make the application as soon after hatching as possible, for at this time the nymphs are more easily killed, and they will be destroyed before the foliage has been damaged. As has already been stated, the later broods are not as a rule so numerous on roses. If they should become destructive, however, the nymphs may be destroyed by repeating the treatment just described.

REPORT

OF THE

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(Connected with Grape Culture Investigations.)

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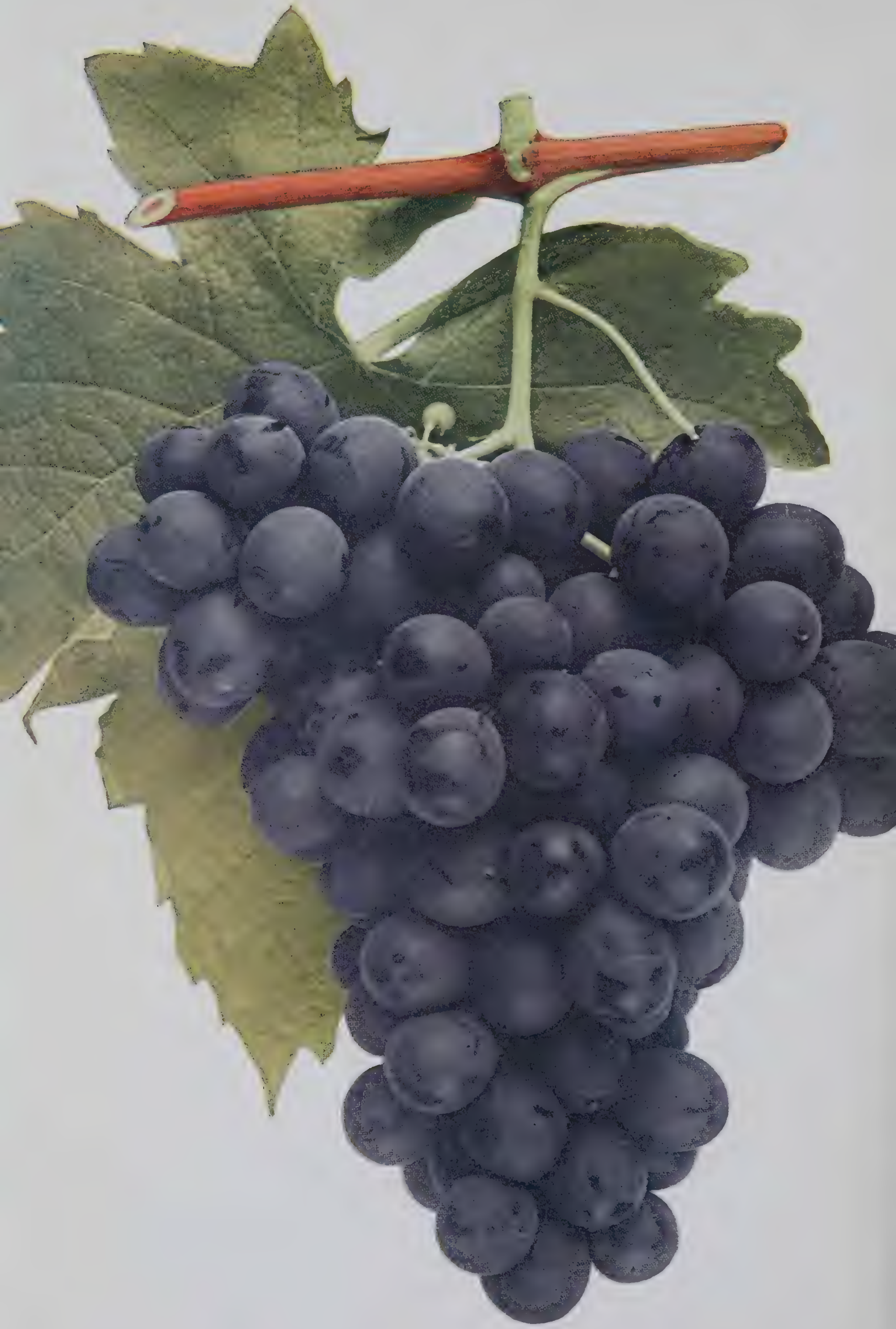

PLATE XLIX.—MUSCAT HAMBURG

(ONE-HALF NATURAL SIZE)

REPORT OF THE DEPARTMENT OF HORTICULTURE.

VINIFERA GRAPES IN NEW YORK.*

R. D. ANTHONY

UNDER DIRECTION OF

U. P. HEDRICK.

SUMMARY.

For two hundred years the colonists and early horticulturists tried in vain to grow the European grape, *Vitis vinifera,* in eastern America. Every attempt resulted in failure until finally the conviction became thoroly established that this grape could not be grown in this region. The end of these attempts was hastened by the discovery of satisfactory native sorts such as Isabella and Catawba.

In the meantime very different results were being secured on the Pacific coast where, in southern and central California, the Vinifera grapes found congenial conditions.

In the century which has passed since the last serious attempt to grow these grapes in the east, we have learned that there were four chief causes for these failures: (1) the downy and powdery mildews, (2) black-rot, (3) a root-sucking louse called the phylloxera and (4) winter injury. Satisfactory means of control have been found for all these troubles.

Experimental culture of the European grape was undertaken at this Station in 1902 when cuttings or plants of 19 varieties were received. In 1911 cuttings of more than 70 varieties were received and grafted upon a miscellaneous collection of Station seedlings ranging from 6 to 10 years old. The results were very satisfactory, most of the plants fruiting in 1913.

By giving the vines winter protection and the usual grape sprays they have been kept in a healthy condition.

As a result of the work at Geneva certain cultural recommendations can be made for New York. One of the most serious difficulties

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* Reprint of Bulletin No. 432, April, 1917.

is to secure plants of the desired kinds. Few can be had from eastern nurseries and not a great number from those in California and then not always on resistant roots. For this reason it will frequently be desirable for the eastern grower to know how to graft cuttings on phylloxera-resistant roots such as ***Vitis riparia.*** This can be done in the nursery row or in the vineyard.

In planting Viniferas less space need be given the vines than with native sorts. Rows six feet apart and plants six feet in the row is a satisfactory distance. Care should be taken that grafted vines do not form roots from the cion.

In the east it is probably best to support the vines with the regular two-wire trellis.

Because of the necessity of bending the trunk to the ground for winter protection, a replacing spur should be left at the base of the trunk to use in forming a new trunk when the old one becomes too stiff. The main trunk should be carried to the lower wire and two fruit canes and two renewal spurs provided for. The young shoots which spring from these canes and spurs grow upright to the second wire when they are pinched off and tied. This gives stockier and more mature canes for the following season.

Cheap winter protection is secured by bending the vines to the ground and covering with a few inches of dirt.

The chief value of the Vinifera grape at present in this State is as a home-garden grape for the amateur, for the commercial grower supplying local markets demanding high quality, and for the plant-breeder seeking to improve the quality of our present varieties.

Most of the Vinifera varieties have originated in regions with a longer season and a much warmer climate than that of New York and many kinds included in the test at Geneva have been discarded because, even in the most favorable seasons, they have not reached maturity.

The varieties are discussed in four groups: (1) Desirable varieties for the grape regions of the State for (a) the table and (b) wine; (2) sorts worthy of testing in the more favorable parts of the State for (a) table and (b) wine; (3) kinds still on probation; (4) varieties of little or no value in the State.

EARLY ATTEMPTS TO GROW VINIFERAS IN THE EASTERN STATES.

The story of the early attempts to introduce the European grape, *Vitis vinifera*, is one of the most interesting chapters in the history of fruit-growing in America — a story which has already been told so well and so often that it is unnecessary here to do more than sketch the main events. In all the accounts left by the early explorers of our Atlantic coast is expressed their surprise at the abundance of grapes growing wild in the woods and thickets. The first colonists were not slow in pressing these into wine for, to them, the vine meant not the table delicacy that it is to us but the sparkling juice which, in those days, seemed so much a necessity of life.

In spite of the many optimistic reports which these early wine-pressers sent back to the Old World, it was soon evident that these wild grapes lacked that clear, vinous flavor so necessary to a good wine grape. For this reason when vineyards came to be planted, cuttings or seeds were sent from France and Germany. For nearly two hundred years the history of these plantations of European sorts is one long series of failures and discouragements. Even in the hands of the most expert vineyardists sent to this country at great expense, the vines soon sickened and died under the adverse conditions of the new country.

One of the last extensive attempts to grow the Vinifera grape in the eastern states was made in Kentucky and Indiana in the early days of the Nineteenth Century by a company of Swiss vineyardists. Many of the best European sorts were introduced but all failed save one, the Cape Grape or Alexander, and all the evidence would indicate that this was a native grape placed in the vineyard by accident.

At about the same time two events took place which changed the whole nature of grape-growing in America — the Isabella grape was discovered in South Carolina and the Catawba in Maryland. Both of these are native grapes and their success marked the end of the first chapter of the story of the Vinifera grape in the east. Since then for nearly seventy-five years, European sorts have been grown only in a limited way by amateurs and then chiefly under glass. During this time the belief that Viniferas could not be grown successfully out of doors in the east became widespread.

It is, perhaps, well to describe some of the differences between the fruit of *Vitis vinifera* and that of our native species. The European grapes have a higher content of sugar and solids, which gives them greater richness and adds to their keeping qualities. Their clear, vinous flavor, frequently with an attractive sprightliness, makes the " foxiness " so strong in many of our native Labruscas seem undesirable in comparison. The greater size of both bunch and berry in many of the foreign kinds adds to their attractiveness. The most noticeable difference is the adherence of the skin and flesh and their greater freedom from objectionable acid or astringency so that many kinds can be eaten whole. In general the Viniferas are more attractive, of richer flavor and better quality and keep much longer in storage.

THE EUROPEAN GRAPE IN CALIFORNIA.

While these grapes were failing so completely in the east very different results were being secured on the western coast. We seldom think of the early Spaniards as bringing anything to Mexico save destruction and desolation, yet some of their most lasting monuments are the missions which they established in the territory which is now the northern part of Mexico and the southwestern states of the Union. These pioneer *padres* were horticulturists of no mean merit and from the mission plantations the Indians learned to grow many European fruits. It was in this way that the Vinifera grape first reached California. Those conditions which caused the failures in the east were lacking here and the mission grapes flourished and spread to become finally the incentive and example which has made California a great grape-growing and wine-making state with 360,000 acres set to vines.

HISTORY OF THE WORK AT GENEVA.

A century has passed since the introduction of the Isabella grape — a hundred years during which science has taught the fruit-grower many things to aid him in his art. We know now that the causes of the early failures with the European grape in the east were chiefly four: (1) The downy and powdery mildews, (2) black rot, (3) a root-sucking louse called the phylloxera which destroys the Vinifera roots but seldom injures the roots of the native grapes, and (4) the inability of this species to withstand the severity of our winters without protection. For all these troubles, satisfactory methods of control have been found, yet, in spite of this, so thoroly have we been taught to believe that Vinifera grapes could not be grown in this region that few have attempted their culture.

An experiment station is always justified in trying the things that " can't be done " when a solution of the problem would seem to be worth while. Such experiments succeed often enough to show that the " impossible " of tradition is purely a relative term.

In the spring of 1902 cuttings of 14 varieties of Viniferas were sent to this Station by W. A. Taylor of the United States Department of Agriculture. These were bench-grafted on cuttings of phylloxera-resistant sorts and planted in the nursery. Lack of proper winter protection caused the loss of all but one variety. The fall of the same year plants of 5 varieties of this species were received from Dr. G. L. Tinker, New Philadelphia, Ohio, who then had a number of European varieties fruiting satisfactorily in a test plantation. Tho we have no statement to that effect, the vines sent to Geneva were doubtless grafted, as Dr. Tinker regularly treated his Viniferas in this way. These six varieties were planted in one of the Station variety vineyards in 1903, in most cases two vines of each kind being set out. Tho two or three vines have died, the remaining ones, after more than thirteen years, are alive and most of them giving good crops.

In the spring of 1910, thru the kindness of George C. Husmann, in charge of the viticultural investigations of the United States Department of Agriculture, a number of grafted vines were sent to the Station. These arrived late in the spring and then were sent to the Vineyard Laboratory at Fredonia in Chautauqua County so that the buds started into growth before planting. This, with unfavorable soil and weather conditions, resulted in the death of most of the plants.

In the spring of 1911, Mr. Husmann sent the Station cuttings of 71 varieties and at the same time Frederic T. Bioletti, Viticulturist of the California Experiment Station, sent cuttings of 30 kinds. At that time a number of vines from six to ten years old in one of the seedling vineyards at the Station had been marked for discarding. Altho these were of miscellaneous origin and contained blood of several species and so were not ideal for such an experiment, it was decided to use them as stocks. They were, therefore, cut off at the ground and cleft-grafted, from two to six seedlings being worked to a single variety. The results were very saisfactory, only two varieties being lost and 70 per ct. of the vines being alive after two years.

The first year the grafts made a vigorous growth. In the fall the shoots were bent to the ground and a few inches of dirt thrown over them. In the spring, as soon as the ground could be worked, they were uncovered and tied to the trellis. With this cheaply applied protection they went thru the winter without injury. Some of the vines fruited in 1912 and since then the Viniferas have, as a whole, borne crops as good or better than most of our native sorts, when allowance is made for the smaller size of the Vinifera vines.

In most seasons the vines have received two applications of bordeaux and lead arsenate spray. No black-rot has been seen in the vineyard and very little mildew. Owing to a severe attack of leaf-hoppers in 1915, it was necessary to give the Viniferas an extra application of a nicotine spray. Except for this, the vines have been given the same cultivation and spraying as the native seedlings growing alongside of them.

CULTURAL RECOMMENDATIONS FOR NEW YORK BASED ON THE RESULTS AT GENEVA.

Propagation.— For some time to come the greatest difficulty in growing Viniferas in the east will be in securing plants of the desired varieties. This Station is, at present, unable to send out either vines or cuttings as all available material is being used in various experiments. Very few eastern nurserymen sell varieties of this species and the kinds which are carried are mainly for growing under glass — too late for outdoor work. At present the best sources are the California nurseries, but even here only a limited list of varieties can be secured and frequently these are not grafted on the desired resistant roots. As soon as ocean freight traffic is restored to a normal condition it will be possible to import vines from foreign nurseries either direct or thru the agency of some New York nursery company.

It is always best to buy rooted vines but sometimes it is necessary to take cuttings in order to secure some much desired variety. This immediately brings up several problems in the handling of cuttings.

PLATE L.— VIEW IN VINIFERA TEST VINEYARD.

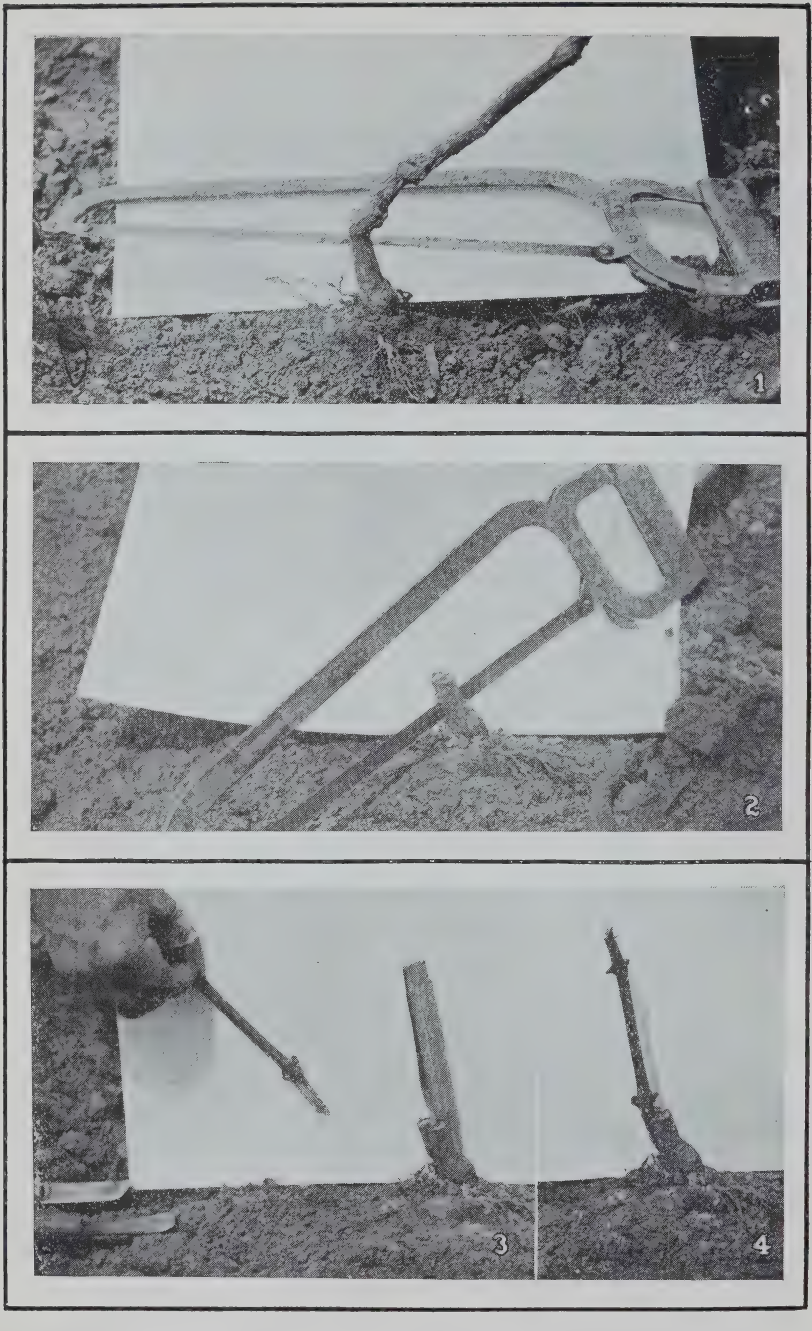


Plate LI.— Grafting a Seven-year-old Vine in Place.

1, Cutting off at ground level; 2, saving the cleft; 3, wedging open cleft for receiving cion; 4, graft completed ready for mounding.

The first is the question of phylloxera-resistant roots. It may be possible to grow Viniferas in many parts of the State upon their own roots without serious damage from the root-louse but the danger of injury is sufficiently great to make it desirable to use resistant roots and with a wise selection of roots there is usually a marked increase in the vigor and yield of the plant. The wild Riparia grape, *Vitis riparia*, sometimes called *Vitis vulpina*, makes a desirable stock in this State. Several selected strains are used, the one called Riparia Gloire being one of the commonest. *Vitis rupestris* is used to a considerable extent in Europe and California and a number of hybrids between these two species have been found of value. A dozen other stocks are used but with our present lack of knowledge it would seem desirable to use those in which the Riparia blood predominates as this species is well adapted to the State, growing wild in most of our woods. The other species commonly growing wild in the east, *Vitis labrusca*, has not proved as valuable for this work; yet many of the good vines in the Station test are grafted on seedlings which are nearly pure Labrusca.

The cions can be whip-grafted either upon cuttings of the desired stock or upon rooted vines of the stock. The work is done in the winter and the grafts are placed under the proper conditions to form a callus before being planted in the spring. These are the common methods of propagation in the nursery but they are somewhat difficult for the amateur as it is an art to secure well callused grafts. The commonest method is to tie the grafted cuttings or roots into bundles and bury them outdoors with the graft uppermost in sand in a protected location, preferably with a southern exposure. Until about a month before time for growth to start, they are kept covered with several inches of sand and frequently the sand is covered with straw. As the weather warms up the depth of the cover is decreased. On cold nights the straw should be put back or a canvas thrown over the sand. During the last four weeks the temperature at the graft should be about 75° F.

A method which is being tried at Geneva and which offers some promise of success is to plant in the nursery row one-year vines of the desired resistant stock as soon as the ground can be worked in the spring. Just before they are ready to start into growth they are cut off at the ground level and whip- or cleft-grafted with a two-eye cion and dirt mounded up to the top of the cion.

For those who can secure desirable cions and who wish to start growing these grapes in a small way, probably the best method is that used at the Station with the cions received in 1911. Vines already in the vineyard but preferably not over six or eight years old are sawed off at the ground just before growth starts. With a thin bladed saw a cleft is cut down thru the center of the stock for about 2 inches. If the grain of the wood is straight this cleft can be split with a chisel. Cions are then cut with two buds and a wedge starting at the lower bud. The cleft in the stock is opened and the cion inserted so that the cambium tissues of the stock and cion — the layer of growth cells between the bark and wood — are in contact. If the stock is large it is best to use two cions, one on each side of the cleft. No wax is used and it is usually not necessary to tie the graft except on young vines. Dry dirt is then mounded to the top of the cion. The purpose of this is to keep the graft uniformly moist and at a more even temperature. Two or three times during the summer the mound should be removed in order to cut off any sprouts which start from the stock or any roots coming from the cion. While this is not a certain method, with proper care there will be but few failures.

A method which a Pennsylvania grower of Viniferas has found very satisfactory is to root the Vinifera cuttings and grow them one year on their own roots; then the vine which is to be used as a stock is planted in the vineyard and the rooted cutting planted beside it so that the shoots from the two may be brought in contact with each other. In June when the plants are in full growth, two vigorous shoots (one from each vine) are brought together and a cut two or three inches long made in each parallel to the length of the cane removing from one-third to one-half of the thickness of the shoot. These flat surfaces exposed by the cuts are then brought into contact with the cambium tissues touching and are tied in place. The tops are checked somewhat by breaking off some of the growth. The following spring the Vinifera roots are cut off below the graft and the top of the stock above the graft is removed.

Planting.— Few Viniferas need be given the space that our native sorts require because of their difference in vigor and in habit of growth. Just what distance will be best under eastern conditions is still a matter of conjecture but probably six-foot rows and the plants the same distance in the row will be satisfactory.

It is not wise to plant grafted vines with the graft much below the surface of the ground as the cion is then liable to throw out roots to such an extent that it becomes practically a vine on its own roots. The plants should be examined occasionally to remove any cion roots that may start.

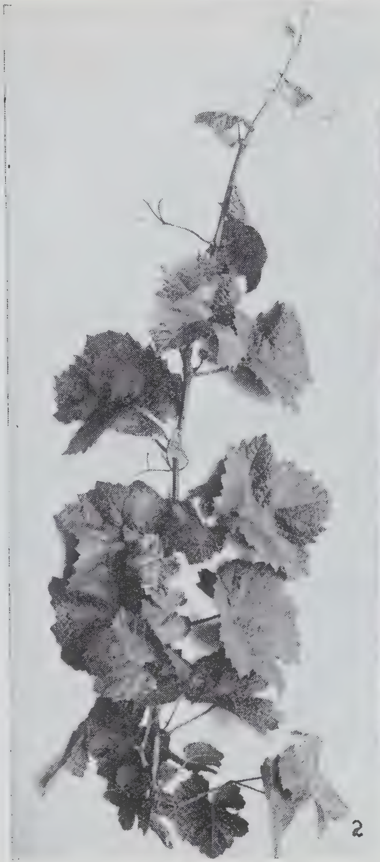
It is necessary to give the vines some support. In the west this is usually supplied by driving a stake at each vine but in the east, at least for the amateur, support can probably best be given by the regular trellis of two wires, the lower wire being about 28 or 30 inches from the ground and the upper wire 54 to 58 inches.

Pruning.— Because of the necessity of bending the trunk to the ground for winter protection, provision should be made for replacing the trunk as soon as it becomes too stiff. This can be done by leaving a replacing spur at the base of the trunk. If two-eye cions are used when the plants are grafted and both buds grow, the shoot from the upper can be used to form the main trunk while that from the lower bud will supply the replacing spur. Each year all but one of the canes coming from this spur are removed and the remaining one cut back to one or two buds until the main trunk begins to be too stiff to bend down readily, then one cane from the spur is left for a new trunk and another cane is pruned for a new renewal spur.

The main trunk is carried up only to the lower wire of the trellis. At the winter pruning, two one-year canes are selected to be tied along this wire, one on each side, and two renewal spurs are left as close to the top of the main trunk as possible. A year later canes which will grow from these renewal spurs are selected for tieing up and new renewal spurs left. For the best production different varieties require different lengths of fruit canes — the one-year canes tied along the wire — but the work at Geneva has not progressed far enough so that any recommendations can be made for particular kinds. The grower will have to watch his vines and prune weak ones heavily and too vigorous ones more lightly. Under normal conditions from four to eight buds are left on each cane, depending upon the vigor of the vine. Some of the older seedlings which were used for stocks in 1911 were so large that two cions were used and in many of such cases where the roots seemed to have sufficient vigor to support the larger top two trunks were formed, one from each graft. By spreading these into a V and making the inner arms shorter, very satisfactory results were secured.

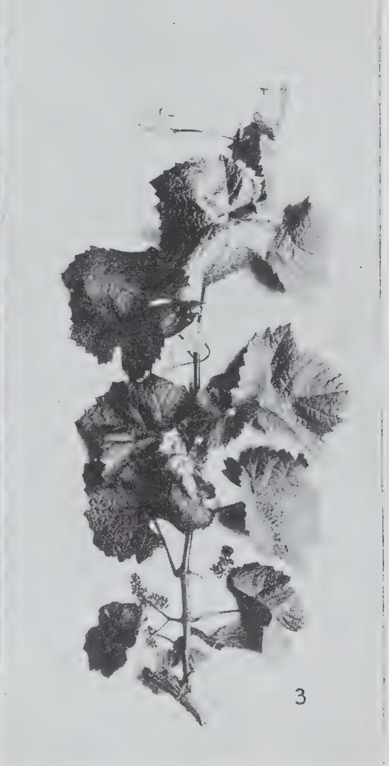
The type of growth of the Vinifera is somewhat different from that of our native kinds. The young shoots which spring from the one-year canes, instead of trailing to the ground or running out along the trellis wires, grow erect. Advantage is taken of this in the pruning system adopted at the Station. The canes and renewal spurs as described above are tied along the lower wire, then the young shoots which come from these grow upward to the second wire. When they are four to six inches above this they are pinched off just above the wire and any which have not already fastened themselves are tied to prevent the wind breaking them off. At the same time if any of the axial buds on the shoots — buds which form at the base of a leaf petiole and which usually remain dormant until the following year but which sometimes start into growth on the young shoots — have begun to form secondary shoots, they are rubbed off, beginning with the node next above the upper cluster and going down to the old cane. This gives the clusters more room and better light. Soon after the first heading-back, the upper buds of the young shoot start lateral growth. These secondary branches usually grow upright and when they are several inches high they are topped off with a sickle. This heading-back results in stockier and more mature canes for the following year and if properly done adds to the fruitfulness of the vine and matures the fruit better.

Winter protection.— A well matured Vinifera is seldom killed outright by the winter even if given no protection but the effect of the first winter is usually to decrease the plant's vitality to such an extent that it is unable to reach proper maturity the next season and so is usually killed the second winter. Wrapping the trunks and canes with straw is not satisfactory and is more expensive than the method used at Geneva. As soon as there is danger of the ground freezing, the vines are pruned, cut from the wire and bent to the ground. Some care has to be exercised in this that the trunk is not broken. While one man holds the vine down two others throw on dirt from each side till it is covered to a depth of five or six inches. During the winter this dirt will settle down considerably and sometimes part of the trunk will be uncovered but no injury has been noted in such cases. This work can be done so rapidly that it adds but a few cents to the cost of growing the vines.



Shoot ready for pruning and disbudding.

Vine ready for summer pruning.



Shoot pruned and disbudded.

Plate LII.— Summer Pruning.

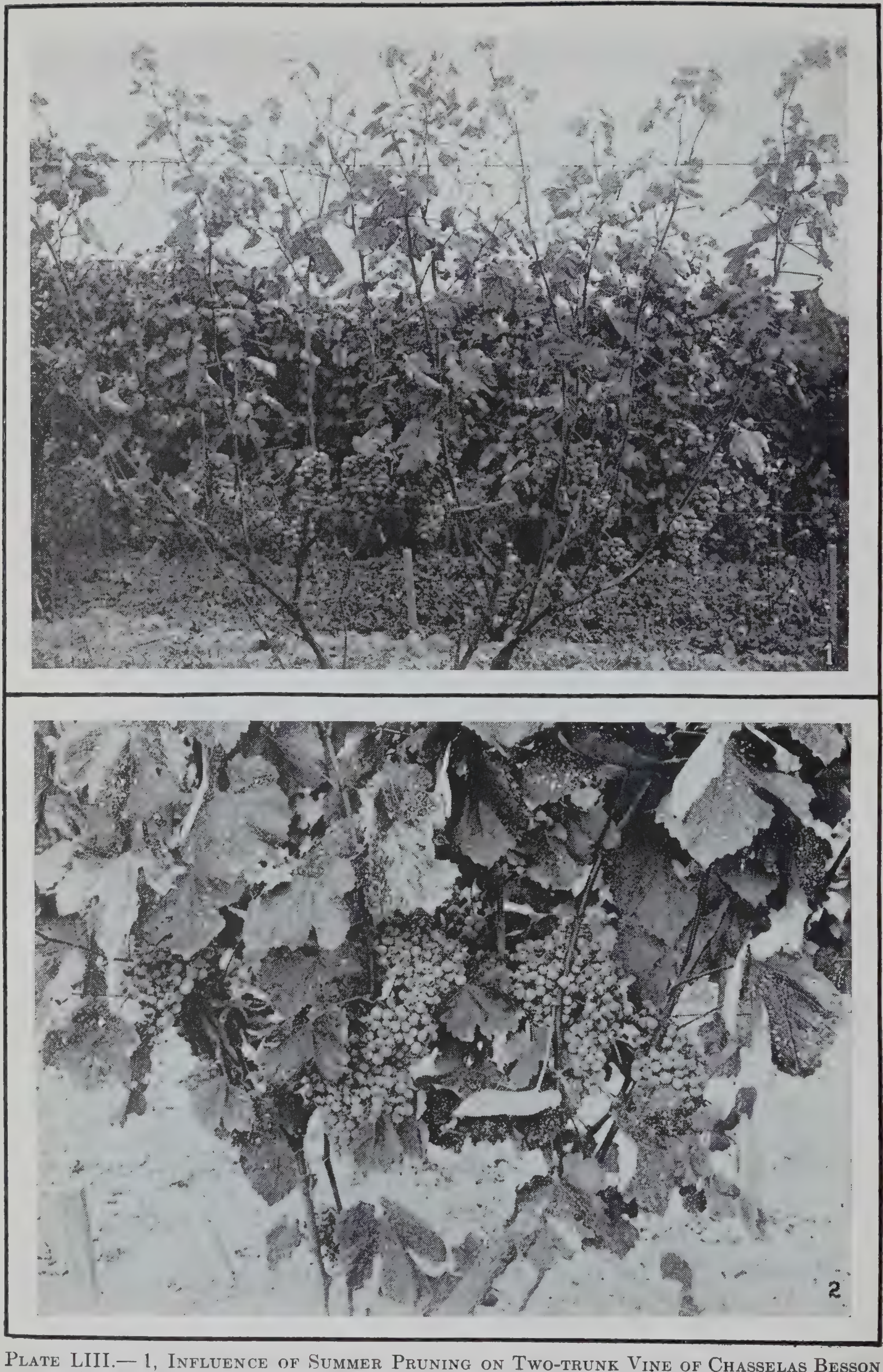


Plate LIII.— 1, Influence of Summer Pruning on Two-trunk Vine of Chasselas Besson (Leaves Partly Lost from Frost); 2, Renewal of Trunk of Calmette Vine.

THE VALUE OF VINIFERAS FOR NEW YORK.

The Vinifera grape should make a most welcome addition to the home-gardens in New York. The rich, clear flavor, the high quality and the size and fine appearance of many of the kinds of this species adapt them splendidly to the home-plantations where quality should be the chief consideration. For the amateur, the lover of good fruit, these can be recommended unhesitatingly. On the other hand, it would not be wise to attempt growing them on a large scale commercially until we have had more experience with them and wider knowledge of the requirements of this species, though the commercial grower who is supplying a high-class local market where good quality counts could well afford to begin testing some of the best varieties to find which are suited to his conditions and to the demands of his market.

The value of the wine varieties in the State is problematic. It will take several years to test out these varieties to find which are best adapted to the conditions in the wine region of the State and, after the proper kinds are found, it will be several years longer before sufficient vines could be brought into bearing to have any effect upon the wine industry. It is a question whether the demand for such grapes would be great enough to justify the expenditure of the time and money necessary to develop them. There are, however, some very good wine sorts which, at the same time, make desirable table grapes and it would be well worth while to begin to test the best of these in the Keuka region.

For nearly a hundred years breeders of grapes have been blending this foreign species with our native kinds and, as a result, of all the grapes which we mark as high in quality, practically every one has some Vinifera blood in it. Altho this hybridization has been going on for nearly a century the work has been desultory and not much more than a dozen European varieties have been used as parents and these chiefly the late-season, greenhouse sorts. There is no other field of fruit-breeding which offers greater promise than the continuation of this work and it is no idle speculation to say that viticulture in New York will be greatly enriched by the Vinifera hybrids which will be introduced in the next fifty years. This Station is already growing several hundred such hybrids.

VARIETIES TESTED AT GENEVA.

Most Vinifera varieties have originated in central and southern Europe or in southern California, regions having a much longer season and a higher summer temperature than at Geneva. Because of this, many kinds tested here will not ripen even in our longest seasons. Others which do well under favorable conditions fail in short, cold seasons. Of the ninety varieties grown here, probably less than ten can be depended on to mature their fruit year after year; but in the grape regions where conditions are more favorable this number can be increased materially, as Geneva is not in a grape region and the climate and soil are so unfavorable for this fruit that only in the best seasons does Catawba reach full maturity.

For two thousand years and more the chief purpose for which the Vinifera grape has been grown was the making of wine and a very large proportion of the varieties now under cultivation have been selected for this use; some of these wine grapes, however, make satisfactory table sorts. The table Viniferas which are shipped into the State in any considerable amount can be counted on the fingers of one hand and unfortunately all of these are probably too late to be of value for cultivation in this State. These facts increase the difficulty of selecting those sorts which will probably prove satisfactory for New York.

In discussing the varieties it has seemed advisable to separate them into four classes: (1) Desirable varieties for the grape regions of the State for (a) table and (b) wine; (2) sorts worthy of testing in more favorable parts of the state for (a) table and (b) wine; (3) kinds still on probation; and (4) varieties of little or no value in the State. The descriptions given below are of necessity brief but whenever possible sufficient characters are described to aid materially in identifying the variety. Vinifera nomenclature is in almost hopeless confusion but when identification was possible the names and descriptions have been compared with one of the latest European authorities.*

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* Ampelographie. P. Viala et V. Vermorel. Tomes 1–7. Paris: 1910.

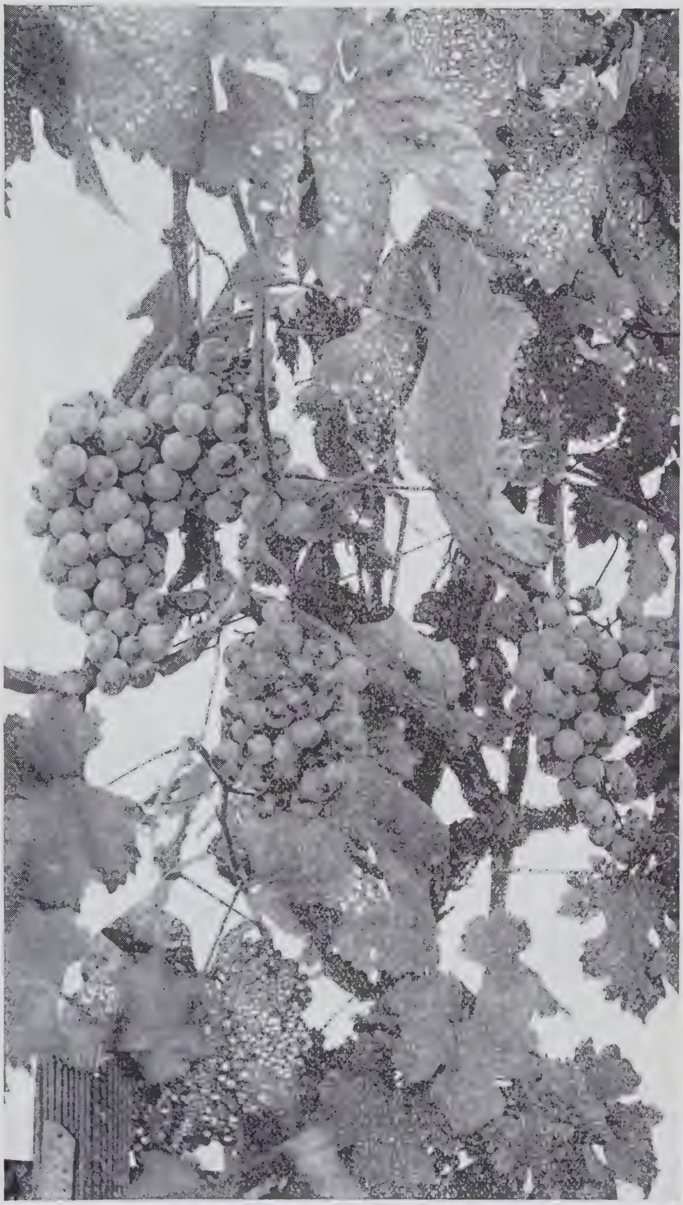

Plate LIV.— Golden Chasselas.

DESIRABLE VARIETIES FOR THE GRAPE REGIONS OF THE STATE.

A. FOR TABLE USE.

Bakator.— This is a Hungarian wine grape but its good quality and early season make it a desirable table grape.

Vine medium in vigor, productive to medium; young leaves tinged red at edges, upper surface glossy; mature leaves large, roundish, upper surface dull, lower surface downy; lobes five, terminal lobe acuminate; basal sinus deep, medium to narrow, closed to overlapping; lower lateral sinus deep, variable in width; upper lateral sinus deep, usually narrows; margins dentate, teeth shallow to medium deep. Flowers appear late; stamens reflexed. Fruit ripens at Geneva the first or second week in October and keeps well in storage; clusters above medium in size, medium in length, broad, frequently double-shouldered, tapering, medium to loose; berries medium to small, slightly oval, light red becoming dark when fully ripe, with thick bloom; skin thin, medium to tender, adherent to the pulp; flesh greenish, juicy, tender, slightly melting, vinous, sweet; quality very good.

The Chasselas Group.— Because of their ancient origin, their earliness, productivity and good quality for the table, this group is the most widely disseminated of all grapes. From this group come three of the promising grapes at Geneva.

Golden Chasselas.— This is the Chasselas Doré of France, the original type of the group. Its age and wide dissemination are shown by the great number of synonyms under which it is known, the one most commonly used in this country in addition to the two given above is Chasselas de Fontainebleau. Among all the cultivated vines none is more widely grown yet it is but a mediocre wine grape and not of the highest quality for the table. Earliness and ability to adapt itself to widely differing environments are its chief recommendations.

Vine medium in vigor, very productive; buds open in mid-season; young leaves tinged with red on both upper and lower surfaces, thinly pubescent to glabrous; mature leaves medium to above in size, slightly cordate, upper surface glabrous, lower surface slightly pubescent along the veins; lobes five in number, terminal lobe acuminate; basal sinus broad and rather deep; lower lateral sinus variable, usually broad and sometimes deep; upper lateral sinus medium to broad and frequently deep; teeth large, obtuse to rounded. Flowers appear late; stamens upright. Fruit ripens early and keeps well in storage; clusters medium to large, medium long, broad to medium, tapering, sometimes with a single shoulder, compactness medium; berries medium to above, slightly oval, pale green to clear yellow, with thin bloom; skin thin, medium tough, adherent, slightly astringent; flesh greenish, translucent, firm to medium, juicy, tender, sweet; quality good to very good.

Chasselas Rose.— This is another grape whose greatest defect is its lack of high quality; in addition it is not as productive as Golden Chasselas. Nevertheless, it is better eating than most of our native grapes and ripens so early for a Vinifera that it is quite desirable. There is some doubt that the variety received under this name is the same as the French Chasselas Violet. The Station also received vines under the name Chasselas Rose Royal. If there is any difference between these two it is that the latter is possibly of slightly better quality.

Vine of medium vigor, productive; opening leaves tinged with red on both surfaces, upper surface glabrous, lower surface glabrous to thinly pubescent. Flowers appear late; stamens upright; mature leaves medium to small, roundish; upper surface medium green, somewhat dull, smooth; lower surface glabrous; lobes three; basal sinus medium in depth and of variable width; lateral sinus medium to deep, narrow; teeth shallow, medium to wide, dentate. Fruit ripens the second week in October and is a medium to good keeper though it loses flavor in storage; clusters above and below medium, long, tapering to cylindrical, medium to compact; berries medium in size, roundish-oval, light red changed to violet-red by the bloom; skin thin, astringent, juicy, tender, sweet, mild; quality medium to good.

Chasselas Besson.— Two vines under this name were sent to the Station in 1902. Because they were not given winter protection for several years, the growth was slow and one eventually died. The other is now vigorous and fruiting well. We have been unable to find anything of the history and distribution of this grape except that it is being grown in test vineyards in California by the United States Department of Agriculture.

Fruit usually ripens the first week in October and keeps very well in storage. Clusters large, compact, tapering. Berries about medium in size, nearly round, green to clear yellow-green; skin medium thick, rather tough, somewhat astringent; flesh translucent, juicy, meaty, sweet, vinous; quality very good.

Feher Szagos.— This is one of the many European sorts whose identity was lost when it was introduced to California and the present name given it. As it grows at Geneva it is rather unattractive because of its dull color and irregular clusters. It has also been somewhat irregular in its bearing. In spite of these disadvantages it is a promising grape as a substitute for Malaga.

Vines vigorous, somewhat uncertain bearers; opening leaves pubescent, red along the edges and a tinge of red on the upper surface. Flowers have upright stamens. Fruit usually ripens the first week in October and does not keep well in storage; clusters large to medium, broad, loose, frequently irregular because of poor setting of fruit; berries large, oval to elliptical, rather dull green, with thin bloom; skin thick, tender, neutral; flesh greenish, translucent, juicy, meaty, tender, sweet; quality of the best; seeds separating easily from the pulp.

Kuristi Mici.— Viala and Vermorel state that there is a Greek grape called Kuristi, but give no description. It is growing in some of the test vineyards of California but apparently is not widely disseminated. Though it has not ripened every year at Geneva it is probably earlier than Catawba.

Fruit ripens the middle of October and keeps well in storage; clusters medium to large, broadly tapering, shouldered, medium loose; berries below to above medium in size, long-oval, green, becoming pale yellow when fully ripe; skin tender, not astringent; flesh juicy, tender, medium meaty, vinous, medium sweet to mildly sprightly; quality good.

Lignan Blanc.— This is a grape whose history probably runs back into the Fourteenth Century and which is cultivated in most of the grape regions of Europe. Of its long list of synonyms, two others are used to some extent in California, Luglienga and Joannenc. No other grape in the Station vineyard, including the native sorts, ripens as early as Lignan Blanc and, though the quality is not of the best, it is far superior to the first native grapes to ripen and promises to be a valuable addition to our list of home grapes. Unfortunately, the birds like it so well that unprotected clusters seldom reach maturity.

Altho the following description does not follow very closely that given by the French authorities, Viala and Vermorel, it seems certain that the vines in the Station vineyards are of this variety.

Vine vigorous, medium productive; buds open early; opening leaves light green, glossy, tinged with red along the edges, thinly pubescent. Flowers appear early for a Vinifera, stamens upright. Leaves medium in size, roundish, somewhat dull green, slightly rugose; lower surface glabrous; blade thick; lobes usually five tho sometimes three; petiolar sinus medium in depth, wide; lower lateral sinus medium in depth, narrow; upper lateral sinus shallow, narrow; margin dentate, teeth long, narrow. Fruit ripens the first of September and is a good keeper; clusters above medium in size, tapering, medium compact; berries medium to large, oval, yellowish-green, with thin bloom; skin thin, tender, neutral; flesh greenish-white, firm, juicy, meaty, sweet; quality good.

Muscat Hamburg.— Altho this variety is well known throughout central Europe, it attains its greatest popularity in England and America where it is the favorite grape for growing under glass. Those who have had an opportunity to taste this rich, musky grape cease to wonder that so many of our present high quality grapes are those with Vinifera blood in their ancestry and our grape-lovers will rejoice in an opportunity to add Muscat Hamburg to their vineyards. Altho it has not reached full maturity every year at Geneva, it seems certain that it will ripen wherever Catawba can be grown. Vines sent to this Station under the names Muscat Albardiens and Moscatello Fino proved to be identical with Muscat Hamburg.

Vines vigorous, productive; buds open late; opening leaves slightly tinged red above and along the edges, pubescent. Flowers appear in mid-season for Viniferas, stamens upright. Foliage large, cordate, five-lobed, terminal lobe acute; basal sinus medium in depth and breadth; lower lateral sinus shallow, narrow; upper lateral sinus deep, narrow, sometimes overlapping; margins sharply dentate, teeth deep. Fruit usually ripe before the middle of October, a medium to good keeper; clusters large, broad, tapering, shouldered, compact to somewhat loose; berries above medium to large, oval, very dark purplish-red, bloom abundant; skin thick, adheres strongly to the pulp, slightly astringent; flesh pale green, translucent, juicy, meaty, tender, sweet, musky; quality very good to best.

Early Black Muscat — Muscat Noir Precoce.—Vines under this name were received from California but the variety is probably an old European one whose identity has been lost as one of the latest French ampelographies makes no mention of such a name. The fruit is very similar to Muscat Hamburg but the two varieties are probably distinct. The berries of Early Black Muscat are slightly larger, more inclined to roundness, and usually darker in color. The season is nearly the same.

Muscat Saint Laurent.— Two white Muscats are growing on the Station grounds. One is widely known in Europe as Muscat Blanc. This is so late that it is of but doubtful value in New York; the other is Muscat Saint Laurent, not so widely known as the former but much earlier and of better quality for a table grape.

Vines medium to below in vigor, medium to productive; opening leaves tinged with red, pubescent to nearly glabrous; mature leaves medium in size, cordate; upper surface somewhat dull dark green, smooth; lower surface glabrous; nodes five, terminal node acuminate; petiolar sinus deep, narrow, overlapping; lateral sinuses varying from deep to shallow; margins crenate, with medium to deep crenations. Fruit ripens the first or second week in October; clusters small, medium to short, tapering to cylindrical, compact; berries small, oval, yellow to green, translucent; skin thick, tough, slightly astringent; flesh yellowish-white, medium in firmness, juicy, tender, sweet, musky; quality very good.

Gray Pinot — Pinot Gris.— The variety known in California as Rülander is the Pinot Gris of France. The grayish color of this grape makes it look unripe and unattractive and so will always make this sort undesirable for market, but its earliness, good quality and rich, sweet flavor make it a favorite home grape.

Vine of medium vigor, productive to very productive; opening leaves pubescent, dull, lightly tinged with red along the edges. Flowers appear early for a Vinifera, stamens upright. Mature leaves medium in size, roundish, thick, upper surface slightly rugose, lower surface glabrous; lobes variable, usually five; petiolar sinus medium in depth, variable in width; lateral sinuses varying from moderately deep and wide to nearly lacking; margins coarsely to deeply dentate. Fruit ripens the first week in October and is an average keeper; clusters small, short, cylindrical, compact; berries medium to small, dull dark greenish-red, giving an unattractive, gray appearance; bloom thin; skin tender, slightly tough; flesh juicy, tender, vinous, sweet, rich; quality very good.

Rosaki.— This is a table and raisin grape of southeastern Europe and Asia Minor. According to some of the California nursery companies it is grown in that state under the name Dattier de Beyrouth, altho it would seem from some French descriptions that there is a separate, very late variety of the latter name. This grape is similar to Malaga and there is a possibility that, in some of the warmer parts of the State, it may be grown commercially as a substitute for the latter.

Vines vigorous, usually very productive; mature leaves large, roundish, rugose, usually five-lobed; terminal lobe acuminate; petiolar sinus moderately deep to deep, medium broad; lower lateral sinus shallow, broad, occasionally lacking; upper lateral sinus shallow to medium, broad; margins broadly and bluntly dentate. Fruit ripens the third week in October, keeping qualities excellent; clusters large, loose, tapering, shouldered; berries large to very large, oval to long-oval, pale yellow-green; flesh translucent, tender, meaty, vinous, sprightly; quality good to very good.

B. FOR WINE.

Most of the table grapes described above are used to a greater or less extent for wine making, tho none of them are of primary importance to the vintner. Unfortunately few of the chief wine sorts will mature at Geneva. Of those mentioned below several offer promise of value for blending with grapes of poorer quality such as Concord to make a superior unfermented juice.

Blue Portuguese.—This is the earliest blue Vinifera on the Station grounds. Altho primarily a wine grape it is pleasant eating but does not keep well when picked and is too well liked by the birds to leave on the vines without protection.

Fruit ripens the latter part of September; clusters medium to small, short, single-shouldered, compact; berries medium to small, roundish-oval, black, with thin bloom; skin tender, thin, slightly astringent; flesh soft, juicy, tender, sweet, vinous; quality good.

Beclan.— This sort has no value as a table grape but would probably make a desirable juice when blended in small amounts with Concord.

Vines very productive; fruit ripens about the middle of October, keeping qualities fair; clusters medium to above, tapering, sometimes shouldered, compact; berries small, roundish to slightly oval, black when fully ripe; bloom medium; skin thick; flesh juicy, melting to slightly firm, sprightly, vinous; quality good to very good.

Kadarka.— This Hungarian grape is rather similar to the black Pinots discussed below but seems to be a better grape for this region. It has no value for the table.

Fruit ripens the third week in October; clusters above medium in size, long, cylindrical to tapering, loose; berries below medium in size, roundish-oval, black, thick bloom; flesh greenish, firm, juicy, tender, slightly stringy, vinous, sprightly; quality good.

Meunier.— This is an old sort, widely grown in Europe even to the northern limit of vine culture. Altho a wine grape it is not to be passed over lightly as a table grape and should be excellent for unfermented juice either alone or in blends.

Fruit ripens the second week in October; does not keep well in storage; clusters medium to small, tapering, medium to compact; berries medium to small, oval, black, appearing blue through the bloom; flesh soft, melting and almost watery except around seeds, vinous, sweet to sprightly; quality good to very good for wine or juice and medium for table.

The Pinots.— This group of grapes is one of the oldest, most important and most widely distributed groups of wine grapes yet some of them are a pleasant addition to the table — one of these we have already described. In addition to the Gray Pinot, three others are growing in the Station vineyards, one white and two black. They are too small and of too light yield to be of much value except where high quality wine grapes are needed.

Black Pinot — Pinot Noir.— This is the type variety of the group and probably the ancestor of most of the other forms. On the Station grounds it is the earliest of the Pinots.

Vines of medium vigor, medium to unproductive; leaves medium to small, roundish, usually three-lobed or, if five-lobed, with shallow, broad, lateral sinuses. Fruit ripens the first or second week in October; clusters small, tapering, compact; brush purple at center; berries medium to small, oval, black; skin medium to thick, tough; flesh juicy, tender, sweet, vinous; quality very good. Seeds large in proportion to the berry.

Pinot de Pernand.— A variety very similar to the Black Pinot, differing only in being more productive, having larger clusters, not as high in quality and considerably later.

White Pinot — Pinot Blanc.— This is believed to be a sport of the Gray Pinot but the fruit at Geneva resembles the Black Pinot more than the gray variety except in color.

Fruit ripens shortly after Black Pinot; clusters medium in size, tapering, compact; berries medium to small, light yellow-green, thin bloom; skin medium to thick, medium to tough; flesh medium in firmness, juicy, vinous, sprightly; quality good.

Teinturier.— If the sale of unfermented juice is extended there will be an increased demand for better quality and finer appearance. All who have sampled such blends agree that the addition of a small amount of juice of certain of our high quality grapes to Concord results in a greatly improved product. Because of its intense, wine-colored juice and good quality, no other Vinifera offers such promise for this purpose as does Teinturier.

Fruit ripens the first week in October, keeping qualities medium; clusters medium to small, rather short and broad, loose; berries small, roundish-oval, black, with thin bloom; skin tough, does not separate easily from the pulp; flesh red, soft, juicy, with wine-colored juice, tender, vinous, sprightly; quality good.

SORTS WORTHY OF TESTING IN THE MORE FAVORABLE PARTS OF THE STATE.

A. FOR TABLE.

There are several of our standard varieties of fruit which are well adapted to the southern Hudson Valley or Long Island which find the season at Geneva entirely too short for them to reach proper maturity. For this reason many of the Vinifera grapes which mature on the Station grounds only in the most favorable seasons would be well worth testing in the warmer parts of the State and would probably be found very desirable there.

Actoni.— The variety grown in California under this name may be the Greek table grape Aetonychi Blanc. It is a very meaty grape of the Malaga type ripening at the end of October.

Clusters large, shouldered, tapering, loose; berries medium to very large, long-oval to oval, clear yellow-green, crisp, meaty, sweet; quality good.

Cinsaut.— This is widely known in France as a wine and table grape and has also been grown to some extent in California for wine. At Geneva it ripens the latter part of October.

Clusters large to medium, broadly tapering, loose; berries large to medium, oval, black when fully ripe; skin tender, thin, not astringent; flesh meaty, tender, sweet, vinous; quality very good.

Early Frankenthal — Frankenthal Precoce.— Altho this variety has not proved very productive in the Station vineyards, it is promising because of its attractive appearance and because it is usually ripe by the middle of October.

Clusters above medium to very large, broadly tapering to blunt, medium compact; berries large, oval, black, medium bloom; skin thin, tender, astringent; flesh tender, medium meaty, juicy, vinous, sweet to sprightly; quality good.

Gradiska.— A number of the California nursery companies are selling this grape for a home table grape and wherever the season is two weeks longer than at Geneva this should be very desirable because of its size, attractiveness, and good quality.

Clusters medium to above in size, variable in shape, loose; berries large to very large, roundish-oval, pale yellowish-green, translucent; flesh tender, meaty, sweet to agreeably sprightly, vinous; quality very good.

Listan.— The variety in the Station vineyard is known in California both as Listan and Palomino yet under these names Viala and Vermorel describe a bluish-black grape while this kind is greenish-yellow. The California Experiment Station in an early viticultural report* described this as an important Spanish white wine and table grape.

Fruit ripens about the 20th of October, keeping qualities good; clusters medium to large, long, single-shouldered, tapering, loose; berries medium to small, roundish, pale greenish-yellow, thin bloom; skin and the adhering flesh medium tender and crisp, flesh surrounding seeds melting; flavor sweet, vinous; quality good.

Mamelon.— Little seems to be known of this variety either in Europe or California, yet it appears to be a productive grape of agreeable flavor which will keep long in storage.

Fruit ripens the latter part of October; clusters large to very large, broad, tapering, compact; berries below medium in size, roundish to roundish-oval, yellowish-green, crisp, tender, sweet to slightly sprightly, vinous; quality good.

Poulsard — Ploussard.— As a wine grape Poulsard is of considerable importance in eastern France and has been grown to a limited extent in California. It makes a desirable table grape, ripening sometimes as early as the 10th of October and keeping well in storage.

Clusters below medium to large, shouldered, tapering, medium loose; berries below medium to large, oval, dark purplish-red to black where exposed to the sun, juicy, tender, melting, sweet, sprightly; quality good.

B. FOR WINE.

The chief wine-producing territory of the State is in the valleys of the western Finger Lakes, Keuka and Canandaigua. The wine grapes discussed below are worthy of testing in those parts of this region where Catawba can be matured with certainty.

Berzamino — Marzemino.— In northern Italy and southern Austria this is an important wine sort and seems to offer special promise for this State because of the coldness of the climate in which it thrives in Europe. It is also rather desirable as a table grape.

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* Calif. Agri. Exp. Sta., Rpt. of the Viticultural work during the seasons 1887-93 with data regarding the Vintages of 1894-95. p. 302.

Fruit ripens from the first to the third week in October; clusters above medium to large, shouldered, tapering, medium compact; brush slightly tinged red; berries small, round, black; flesh melting, juicy, sprightly; quality very good.

Calmette.— Very similar in its fruit characters to Teinturier, Calmette differs from the latter in being more productive, a week or more later, having soft, melting flesh and poorer quality.

Gray Chauché — Chauché Gris — Chaouch.— A casual inspection would lead one to mistake this for the Gray Pinot as clusters of the two are much the same size and shape and both have the same peculiar color which has led to their being called "gray." Gray Chauché is, however, not as good as Gray Pinot, the skin is rather tough and astringent and the flesh is not as sweet nor as high in quality.

Franken Riesling.— This is not the famous Riesling of the Rhine Valley — one of the best known grapes of Europe — but it is a widely cultivated sort of central Europe where it is commonly known as Sylvaner. At Geneva it is a heavy producer and usually ripens by the middle of October. When fully ripe it is pleasant eating. One of the vines in the Station vineyard is now 14 years old and very productive and healthy.

Clusters medium to below, slightly tapering, compact; berries medium to below, oval, yellowish-green; bloom thin; skin tender, slightly astringent; flesh medium firm, tender, melting around the seeds, vinous, sprightly; quality very good.

Large Sauvignon — Sauvignon Vert.— This variety is well liked as a wine grape especially for champagne both in France and California. When fully ripe it is pleasant eating.

Clusters below to above medium in size, tapering to cylindrical, compact; berries medium to small, oval, green to yellowish-green; flesh tender, medium melting, sweet, mild; quality good.

Slankamenka.— In Hungary this is an important white wine grape but it has little value for the table.

Fruit ripens about the 20th of October; clusters medium to large, broadly shouldered, tapering, compact to very compact; berries medium to below in size, oval, greenish-yellow; skin thick, meaty, slightly astringent; flesh juicy, soft, tender, mild, sweet; quality very good.

Syrah — Petit Syrah.— This old French grape is a popular variety in California for red wine. It has no value as a table sort.

Fruit ripens at the end of October; clusters medium to above in size, long, tapering, compact; berries below medium in size, oval, black; flesh juicy, melting, sprightly; quality good.

KINDS STILL ON PROBATION.

For one cause or another there are a number of varieties whose value is still uncertain. They show too many qualities of value to be discarded at present but their record is either too incomplete or is not sufficiently satisfactory for them to be recommended. As the test progresses some of them will, beyond doubt, prove desirable but many will be marked for discard.

Carignane.— A sprightly, bluish-black, wine grape; probably too sprightly for a table grape.

Fruit matures late; clusters below to above medium in size, compact; berries medium to above in size, meaty.

Chardonnay — Chablis.— Widely grown in northern France and southern Germany; resembles White Pinot but is two weeks later.

Clusters small, very compact; berries small, roundish oval, green, vinous, sprightly.

Lahn Traube — Van der Laan Traube.— A Holland and German grape much like Golden Chasselas but ripens two weeks later. May make a desirable table grape.

Malaga.— This is the large, oval, yellowish-green grape so commonly received in our markets from California. It is probable that it would reach a satisfactory degree of maturity in the southern Hudson Valley.

Mammolo Toscano.— It is probable that this is the Refajone Nero, a wine grape of Tuscany.

Fruit matures late; clusters large, several-shouldered, compact; berries above medium in size, purplish-black, roundish-oval, meaty, agreeably sprightly; promising as a table grape.

Mantuo de Pilas.— There is some doubt that the variety received under this name is true to name as it seems to ripen earlier than the European variety of this name is reported to mature.

As grown here the clusters are above medium in size, cylindical, medium compact; berries medium to above in size, oval, pale green, meaty, sweet; desirable for the table but needs a long season.

Monica — Canaiolo.— Of value only for wine.

Clusters large, broadly tapering, medium compact; berries above medium in size, oval, black; skin tough; flesh melting. Ripens at the end of October.

Muscat of Alexandria.— This is extensively planted in California for a raisin grape and is usually known there as Muscat. Would probably make a desirable table grape in the southern Hudson Valley.

Black Hungarian Muscat — Muscat Noir de Hongrie.— Similar to early Black Muscat but seems to be later.

Large Early Black Muscat — Muscat Gros Noir Hatif.— Much like Muscat Hamburg but not as good in quality and is somewhat later.

Oliver de Serres.— This is a very attractive, large, firm, black grape with a sweet, vinous flavor and very good in quality. Ripens the last of October and is a good table grape.

Quagliano.— Very similar to Poulsard but not as good nor as early.

Refosco.— Of the type of Black Pinot but more productive, larger and one to two weeks later. Of value for wine only.

Savagnin Rose — Gewurztraminer — Red Traminer. — In cluster and berry this looks much like the Delaware. Flavor spicy and pleasing but the skin is too tough. Ripens the last of October.

Semillon.— A small, pale green grape of good quality, but probably too unproductive and too small to be of value.

Servan Blanc — Servant.— As a table grape this seems promising for the warmer sections of the State.

Vines productive; clusters above medium to large, shouldered, tapering, medium to compact; berries medium to large, oval, pale yellowish-green; skin tender; flesh translucent, meaty, sprightly. Considerably like Gradiska.

Steinschiller — Rother Steinschiller.— Like Savagnin Rose this variety looks like Delaware. It is pleasant eating but ripens unevenly and is rather unattractive.

Sultanina.— One of the important California grapes is the greenish-yellow, seedless raisin grape, Thompson Seedless. This is the Sultanina of Greece and the Orient. There is also a light red form called Sultanina Rosea. Both of these can probably be matured in the warmer parts of the state and will make a pleasant addition to the home vineyard because of their good quality and their lack of seeds. The berries are small but the clusters are large.

Valdepenas.— This is a wine grape which may be worth testing as a table grape in the warmer parts of the grape regions.

Clusters large to very large, shouldered, tapering, very compact; berries medium to below in size, roundish-oval, black, firm, meaty, crisp; quality good to very good.

Zinfandel.— Widely grown in California as a wine grape; of no value for the table.

Clusters too compact and the flesh too tender to handle easily. Worth testing as a wine grape because of its productivity and good quality.

VARIETIES OF LITTLE OR NO VALUE IN NEW YORK.

Because of the short growing season in New York as compared with that of most of the Vinifera regions many sorts will probably not mature even under the most favorable conditions. These are mentioned below.

Angelino.— A large, dark red grape.

Aramon.— Might possibly ripen on Long Island.

Clusters very large and compact; berries large, roundish, probably black when fully ripe.

Black Alicante — Black Saint Peter.— This is an important table grape in England and northern France. The vines at Geneva may not be true to name as here it is very late and the quality is poor.

Clusters long, tapering, medium compact; berries above medium in size, roundish, black; flesh tender, rather tart.

Black Damascus.— A large, oval, black grape with meaty, vinous, sprightly flesh. Would probably mature on Long Island.

Black Morocco — Ribier.— Clusters very large, loose; berries very large, oval, dark purplish-red.

Black Muscat — Jura Muscat.— As compared with Muscat Hamburg the clusters are more compact, the berries smaller and not as dark, the flavor is much stronger of musk, the quality is poorer and it ripens a week later.

Cornichon Violet.— A very attractive berry, very large, long, blue-black, meaty. Needs too long a season.

Elbling — Burger.— Widely grown in Germany for wine and might be of some value in the lower Hudson Valley for this use.

Clusters above medium to very large, long, compact; berries medium to above in size, roundish-oval, yellowish-green, flesh tender, stringy, sprightly.

Ferrara.— A large, purplish, table grape.

Flame Tokay.— Grown in California for shipping. A large, red grape. Would probably ripen on Long Island.

Fintendo.— Supposed to be a Spanish table grape but of no value here except for wine.

Clusters large to very large, tapering, very compact; berries medium, oval, black, tender, vinous, sweet; brush tinged red.

Green Hungarian.— Productive. Might make a good wine grape.

Clusters large, tapering, compact; berries above medium in size, round, clear green; flesh tender, mild to sprightly.

Grenache.— A late wine grape, of no value for the table.

Clusters large, shouldered, tapering, compact; berries below medium in size, oval, purplish-black; flesh melting, juicy, tart.

Malvasia.— In Europe this name is applied to a number of grapes. The variety growing at Geneva under this name is somewhat like Early Frankenthal, but is later, not as firm and more sprightly.

Malvasia Rosario.— Similar to the Malvasia discussed above but much firmer.

Millennium.— Productive and may be worth testing further.

Clusters of medium size, compact; berries medium in size, oval, yellowish-green; flesh medium tender, sweet; quality not high and skin thick and tough. Ripens the latter part of October.

Pedro Ximines — Pedro Jimenes.— Altho a wine grape this would be desirable for the table if fully ripe.

Clusters large, loose, tapering; berries large, oval, yellowish-green, medium meaty, sweet, vinous.

Schiradzouli.— A table grape with large, long, firm, purplish-red berries.

White Muscat — Muscat Blanc — Muscat Frontignan.— Similar to Muscat Saint Laurent already described, but later and with a much stronger muscat flavor and poorer quality.

WINTER INJURY OF GRAPES.*

F. E. GLADWIN.

SUMMARY.

The data show that approximately half the fruit buds in Concord vineyards about Fredonia and in particular on the Experiment Grounds were killed during the winter of 1909–1910.

The injury can be traced to a lack of maturity of the tissues as a result of the sudden termination of the maturing period on October 12, 1909, by unseasonably low temperature.

Maturity is indicated by small amounts of water in the tissues and by thickening of the cell walls. Immaturity is therefore favored by high temperatures and abundant rainfall during late summer and early fall.

The embryo flower clusters may succumb to low temperatures if they enter the dormant period immature and yet the foliage of the bud expand normally.

Light crops in years following heavy yields are probably due in part to injury to the floral parts by cold; but unless the extent of the injury be considerable, winter killing is overlooked in explaining the light yield.

Each year some buds fail to reach complete maturity and their failure at foliation is generally ascribed to some other agency.

The most completely matured buds on a cane begin with the fifth or sixth from the base of it and extend to within six or eight buds from the tip. The basal buds are usually small and poorly ripened, likewise those near the apex.

The low temperatures of December, 1909, probably killed the buds that were to bear the crop of 1910; but freezing temperatures after April 6, 1910, may also have caused injury or increased it.

Leafing with some native varieties occurs after ten or twelve days having a daily mean temperature of 52 to 53 degrees F.; and

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* Reprint of Bulletin No. 433, April, 1917.

from March 18 to April 6, 1910, nineteen days, a daily mean temperature of 53.2 degrees was recorded. Following this period came freezing temperatures, and injury may have resulted therefrom to the prematurely stimulated buds.

Immaturity of grape buds and canes has been noted after temperatures of 10 to 12 degrees below the freezing point in the fall, notably in 1912.

Winter bud-injury during 1915–1916 ranged from 10 to 100 per ct. among the varieties growing on the Station grounds at Fredonia. Injury to Concord varied from 19 to 45 per ct. at the same place. The killing was general thruout the "Grape Belt."

Unfavorable climatic conditions existed during the maturing months of August, September and October, 1915, as indicated by the poor ripening of the fruit of 1915. The lack of ripeness is shown by the sugar and acid content.

During the week of January 22–29, 1916, the average hourly temperature for 96 consecutive hours was 52.6 degrees, which no doubt awakened growth activity at this time; while the minimum temperature of the winter,—16 degrees, occurred in March. The winter killing of 1915–1916 may have occurred, therefore, either shortly after the high temperatures of January, or not until the low ones of March.

Considering the immaturity of the tissues at the beginning of the dormant period, it is probable that the injury would have occurred regardless of the unseasonable temperatures of January.

The fertilizer elements, nitrogen, phosphorus and potassium, did not influence maturity and hence their effect was not apparent as influencing the degree of killing.

Extensive injury is closely correlated with poorly drained soils, altho bud killing occurred where the drainage conditions were satisfactory.

Severe pruning after late frost injury of spring has apparently indirectly favored bud killing thru inducing rank wood growth.

Maturity of bud and wood is probably correlated with the ripeness of the fruit, as determined from sugar analyses of the fresh juice.

Resistance to low temperatures is probably a species character and is possibly correlated with the hardness of wood.

INTRODUCTION.

It is a general belief among vineyardists that the bearing of a large crop one season necessarily curtails the yield of the year following thru exhaustion of the available plant food elements. In part this is doubtless true, but a short crop after a heavy one is determined to a large extent not thru food exhaustion alone, but thru the imperfect maturity of cane and bud following the heavy crop year. Immaturity may be so evident at the beginning of winter that a large number of the immature canes are pruned away, yet at foliation in the following spring many other buds fail to start.

Again, canes and buds may appear well matured at the fall pruning and yet varying numbers of buds fail to open at the starting of growth in the spring; or make a small beginning, only to die later. If during the winter there occurred a period of unseasonably high temperatures, the failure at foliation is ascribed to low temperatures following the warm spell, or if winter temperatures were unusually low, and dead or injured buds are noted in the spring, the trouble is dismissed as winter killing without regard to the factors that made the killing possible. It is but rarely that all buds reserved for fruiting break at foliation, and while small deficiencies are usually overlooked they are sometimes ascribed to accident; yet the same factors were probably operative in years of negligible bud failure as in the year of extensive injury. The difference is only one of degree.

During the past eight years there have been three seasons in which Concord buds did not reach complete ripeness or maturity, 1909, 1912 and 1915. The crop immediately following each of these years was very light.

In order to show the relationship of climate to maturity and finally to the extent of injury it will be necessary to trace in detail prevailing weather conditions month by month for several years.

The literature dealing with winter injury either to the wood or buds of the grape is very limited, not alone in the United States but also from foreign sources.

The following presentation of the findings of various investigators dealing with winter killing of plants other than the grape, is brought forward, with the assumption that the causal agencies operate in the same manner in either case, tho the susceptibility to unusual climatic changes may not be the same with all fruits. It is quite apparent that the vine is very sensitive to changed growing conditions, and as the time elapsing between foliation and the beginning of the rest period is relatively short as compared with many other fruits, unfavorable weather changes probably bring about very pronounced changes, both in the fruit and wood of the vine.

Much of the controversial literature has been omitted as it can serve no purpose in an understanding of the data that is here presented.

In a search of the writings of others on the subject of variety resistance to winter injury, reference was found to only a few sorts, and with these the injury was largely confined to root and stem.

LITERATURE CONCERNING SUSCEPTIBILITY OF PLANT TISSUES TO COLD AND THE CONDITIONS THAT FAVOR WINTER KILLING.

IMMATURITY AND INJURY.

English[1] states that the reason a twig is found in an immature condition in the winter is because the cold occurred in the autumn before the plant had normally completed its summer growth. Further, the terrible effect of the cold on the plant world during the winter of 1879–1880 can be attributed to the long continued freezing combined with the condition of the plants when winter set in. The climatic conditions of the summer and autumn of 1879 were such as to cause the wood of many trees and shrubs of that summer's growth to be in an immature condition at the beginning of winter.

Chandler[2] concludes that the most important feature affecting the hardiness of plant tissue is maturity; that is, the condition of resistance that plants reach during the winter dormant period. Maturity in the case of cambium may be intimately associated with the process of drying out. Of the tissue above ground during the periods when the most complete maturity is reached, the most tender parts are the pith cells and the fruit buds.

Beach and Allen[3] state that the maturity of wood at the time cold weather sets in undoubtedly has a most important bearing upon the ability of the tree to withstand cold and that by noting the formation of terminal buds and the time of leaf fall a pretty accurate idea of maturity can be secured, except in case of a few varieties. The hardier varieties on the average had a slightly lower moisture content than the more tender varieties.

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[1] English. Winter injury of twigs and buds. Univ. of Vermont. Thesis.

[2] Chandler, W. H. The killing of plant tissue by low temperature. Mo. Expt. Sta. Res. Bul. No. 8. 1913.

[3] Beach and Allen. Hardiness in the apple as correlated with structure and composition. Iowa Sta. Res. Bul. 21.

DEATH OF PLANT TISSUE THRU FREEZING.

Goeppert[4] claims that the death from freezing actually occurs during the period of the low temperature.

Sachs[5] says, "It is after thawing and due to the manner of thawing."

Whipple[6] concludes, "In most cases when the tissues of plants are actually frozen, the immediate effect is to draw the moisture out of the cells and freeze it as ice outside the cell walls or in the intercellular spaces.

English[7] states "That under the influence of cold, water escapes from the cells, and may be frozen either in the space between them or on the surface of the exposed parts, and it is frequently noticed that a dry summer, followed by a warm moist autumn, leaves the plant in a poor condition to withstand the winter."

Goeppert[4] maintains that the wind is involved in the killing back of shoots and branches of trees, especialy that from the north thru causing excessive evaporation, thus resulting in a drying out of the tissues.

Bernbeck[8] subjected various plants to wind rates as high as 31.3 miles per hour and obtained some very interesting results. He found that transpiration, or the giving up of water to the air, was proportional to the wind rate and to the amount of bending or swaying undergone by the different parts of the plant, and that the excessive loss of water ceased when the bending was eliminated.

Gorke[9] found that when the plant sap is frozen, certain proteids may be precipitated out, and apparently those plants that are most easily killed by freezing have their proteids precipitated out at the highest temperatures.

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[4] Goeppert. Ueber die Warmeentwickelung in dem Pflanzen: deren Gefrieren und die Schutzmittel gegen dasselbe. Book. 1830.

[5] Sachs. *Ber u. d. Ver. d. Kgl. Sachs. Gesell d. Wiss.* 12:1–50. Leipzig, 1860.

[6] Whipple, O. B. Winter injury to fruit buds of the apple and pear. Mont Sta. Bul. 91.

[7] English. Winter killing of twigs and buds. Univ. of Vermont. Thesis.

[8] Bernbeck, O. Der Wind als planzenpathologischer Faktor, Dissertation Bonn. p. 116. 1907.

[9] Gorke. *Landw. Vers. Stat.*, 65:149. 1906.

RATE OF FREEZING AND THAWING AS RELATED TO WINTER INJURY.

Nearly all investigators agree that a rapid decline to a certain low temperature is more injurious than a gradual lowering to that temperature. This is reasonable, as root absorption of water with a frozen soil can not expand simultaneously with the sudden removal of water by evaporation from the cells, induced by the rapid decline to a low temperature.

Whipple[10] says, " If the freeze be not too severe or of too long duration, and the tissues thaw gradually, much of the moisture withdrawn from the cells may be re-absorbed by the cells, and no ill effects result. If the freeze extend over a period of time the buds may freeze dry as does a wet cloth on a cold day. If severe low temperatures be accompanied by high winds, from a dry quarter especially, the buds are in greater danger of freezing dry, and when dried to a certain point, death or a more or less permanent injury results. However, the tissues of plants do not freeze when the temperature reaches 32 degrees F., as the cell sap is much more concentrated, hence the temperature of the air must reach several degrees lower than 32 degrees, the freezing point of water."

It has been stated by other investigators that dormant buds require zero temperatures before ice begins to form within the tissues.

DO BUD SCALES PROTECT FROM LOW TEMPERATURES?

Chandler[11] states, " That the scales of peach buds do not serve to protect them from low temperatures. Buds frozen in the Laboratory with the scales removed were slightly more resistant than were the buds with the scales not removed."

English[12] however concludes, " That warm weather if long continued may cause the buds to swell, though not preceptibly so, and when the bud scales have spread apart a little, thus permitting the penetration of cold more readily, if a cold snap of several days follows, the bud is very likely to be injured."

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[10] Whipple, O. B. Winter injury to fruit buds of the apple and pear. Mont. Sta. Bul. 91.

[11] L. c.

[12] L. c.

EFFECTS OF LOW TEMPERATURES UPON THE VARIOUS PARTS OF THE PLANT.

Whipple [13] suggests that winter-injured fruit buds of the apple, when the killing of the buds is not complete, may develop leaves, and that the so-called off years are in reality such by reason of this partial injury.

Chandler [14] states that usually when peach buds are exposed to low temperatures the first tissues to be killed are the flower parts in the bud. If the temperature goes still lower, the pith of the bud may be killed and even the pith at the base of the buds.

Waite [15] reports that the fruit buds of peach are apparently more hardy than the youngest, tender sap wood, and that the hardiest portion of the tree is the cambium layer.

Cuboni [16] found that the cut surfaces around the juncture of the buds with the tissues of the canes, are not as compact as in the uninjured tissues, but are furrowed with transverse or radial lines. These when examined with the lens correspond to the fissures and crevices produced by freezing.

The presence of such fissures and crevices is a characteristic, distinguished easily with the naked eye, and constitutes a sure indication of the marked alterations produced by cold. Buds which show these characters are certainly dead.

EFFECTIVE INITIAL TEMPERATURES FOR THE STARTING OF GROWTH ACTIVITY IN THE VINE.

De Candolle [17] states that the vegetation of the vine starts at a minimum of 50 degrees Fahrenheit. Durand [18] has found the same initial temperature to be effective for Vinifera varieties. The minimum temperature at which the vine commences foliation, according to Count de Gasparin [19] is 49.1 degrees F. Boussingault [20] from observations made at Strasburg observes that a mean temperature of 45.5 degrees F. is required. Lippincott[21] states this to be true likewise of American varieties. To quote, " At this date (April 24, 1864) the buds of Concord are much swollen, some of them have opened, and several other varieties are in various stages of enlargement but without showing the leaf."

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[13] L. c.

[14] L. c.

[15] Waite, M. B. Fruit trees frozen in 1904. U. S. Dept. of Agr. Bur. of Plant Industry. Bul. No. 51. Pt. III.

[16] Cuboni, G. Gli effetti del gelo sui tracli e le gemme del le viti. *Staz. Sper. Agr. Ital.* 26:1894.

[17] de Candolle, A. Geographic botanique raisonnee.

[18] Durand, E. The quantity of heat required by grape vines. *Vigne Amer.* **21. 1897.**

[19] de Gasparin, Count. Cours d'Agriculture.

[20] Boussingault. Rural Economie.

Observation[22] made at Waterloo, N. Y., 1862, exhibited the fact that Diana and Clinton began the leafing process at 51.66 degrees F. for 10 to 12 days previous, or from the first of May, and that other varieties opened their leaves under a mean of 53.13 degrees beginning with the same date."

VARIETAL RESISTANCE TO LOW TEMPERATURES.

Selby[23] says, " The principal reason why some varieties of apples suffered worse than others from freezing is found in the difference in the maturity of the growth."

Taft and Wilken[24] reporting on injury to grapes in Michigan during the fall and winter of 1906–1907 state that there was no difference noted in the resistance of varieties to the freeze. There was no fruit except on Worden, which had protection from neighboring trees.

Eustace,[25] writing of the injury from cold during the winter of 1903–1904, states the greatest damage was in the Hudson Valley where more than 40 degrees below zero was recorded. "One of the most striking differences was seen in a vineyard at Marlboro. There were two varieties, Niagara and Worden. All the former were killed to the snow line while none of the latter seemed to be seriously injured. The vines were of the same age and all conditions as to soil, altitude and care were the same. In this instance the difference in injury was purely due the variety."

It is a well known fact that Niagara is particularly tender in bud and root.

Quaintance[26] reports that the low temperature, —7 degrees of February 13, 1899, occurring in Georgia, resulted in but slight injury to grape vines as they were practically in a dormant condition; however varieties of the species Rotundifolia were injured. All of the Eden variety were killed to the ground, while three Scuppernong soon died as a result.

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[21] Lippincott, James S. Geography of plants. Rpt. Com. Agr. U. S. D. A. 1863.

[22] Brehm, *Horticulturist.* 18:52–53.

[23] Selby, A. D. Fall and early winter injuries to orchard trees and shrubbery by freezing.

[24] Taft and Wilken, Rpt. So. Haven Sub Sta. 1907. Mich. Sta. Spec. Bul. No. 46. 1908.

[25] Eustace. Winter injury to fruit trees. N. Y. Sta. Bul. 269. 1905.

[26] Quaintance, Rpt. of Horticulturist. Georgia Station Rpt. 1899.

Beach and Allen [27] in a study of " Hardiness in the apple as correlated with structure and composition " found that the hardness of the wood of certain varieties was quite closely correlated with hardiness or resistance to low temperatures."

WEATHER CONDITIONS OF 1908–1910 AS RELATED TO WOOD AND BUD MATURITY OF 1909 AND THE WINTER INJURY THAT FOLLOWED.

The 1908 grape crop was very light. The temperature for the growing months May, June and July, as well as the precipitation, was approximately normal for this region, while for the maturing period of August, September and October there was an excess of 95.6 degrees above the normal, the precipitation being 5.89 inches below the usual amount with 5.23 inches of this total deficiency occurring in September and October.

Table I gives the temperatures and precipitation for the growing and maturing periods of 1908.

TABLE I.— SUMMARY OF TEMPERATURE AND PRECIPITATION, MAY-OCTOBER, 1908.

MONTH.	TEMPERATURES.		PRECIPITATION.	
	Daily mean.	Departure from normal.	Total for month.	Departure.
May	58.2 Deg.	+ 1.4 Deg.	5.54 in.	+ 1.91 in.
June	67.3 "	+ 1.7 "	3.18 "	+ 0.10 "
July	70.2 "	— 0.8 "	2.06 "	— 2.46 "
August	60.9 "	— 1.8 "	2.5 "	— 0.66 "
September	66.6 "	+ 3.6 "	0.6 "	— 2.88 "
October	54.0 "	+ 1.4 "	1.35 "	— 2.35 "

These records are taken from the Cooperative Station of the U. S. Weather Bureau at Westfield. The excess and deficiency recorded represent the departures from the normal.

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[27] Beach and Allen. Hardiness in the apple as correlated with structure and composition. Iowa Sta. Res. Bul. 21. 1915.

The winter months of 1908–1909 were approximately normal so far as precipitation is concerned, while the five months November to March, inclusive, had an excess of 321.9 degrees of heat over the normal amount for the period.

The favorable weather conditions of the season of 1908, coupled with a light crop, resulted in well matured fruit and wood, with a greater amount of the latter available for fruiting purposes in 1909 than had been attained for several years previously. With very moderate temperatures prevailing thruout the winter, and the precipitation approximately normal, the fruit buds came thru to spring uninjured and promised a large crop for the season of 1909.

Table II summarizes the meteorological data with special phenomena affecting maturity of wood and the grape crops of 1909–1910. The season of 1909 was characterized generally by unfavorable climatic conditions. The month of May was cool and with a rainfall slightly above the average. The first half of June was cool and wet, while in the latter half the temperature rose, making the daily mean for the month a little above normal, and a drouth began that continued thru July. The mean temperature for July was also decidedly below normal. Low temperatures continued during August but late in the month the drouth was broken making an excess of rainfall equal to the deficiency for July. The lowering of temperatures was intensified in September, and 2.5 inches more of rain fell than is usual for this month. The average daily mean temperature for October was 6.6 degrees below normal.

Tuesday, October 12th, began very cold and this increased during the day, with a strong northwest wind blowing continuously. During the night of the 13th and 14th ice had formed to a considerable thickness and the road froze dry. This condition prevailed thruout the day. A second freeze occurred on the night of the 19th, tho not as severe as the earlier one. Heavy rainfalls had been recorded for the preceding six weeks. At the time of the first freeze the grape harvest had not been completed in many vineyards. In some, where the foliage was scant, the berries were frozen hard. In others, protected by bordeaux sprays, no appreciable damage was done owing to the persistence of the foliage. One factor that was very apparent in lessening and even preventing entirely the disastrous effects of the cold spell, is the bounding ridge that determines the Chautauqua and Erie Fruit Belt. At the Vineyard Laboratory

TABLE II.— SUMMARY OF METEOROLOGICAL DATA, WITH SPECIAL PHENOMENA, AFFECTING THE MATURITY OF WOOD AND THE GRAPE CROPS OF 1909 AND 1910.

MONTH.	TEMPERATURE.			PRECIPITATION.			Remarks.
	RANGE.			RANGE.			
	Daily excess.	Daily deficiency.	Extremes.	Monthly excess.	Monthly deficiency.	Extremes.	
1909							
May		1.3 Deg.		Slight			
June	0.2 Deg.				1.44 in.		
July		3.7 "			1.65 "		
August		1.3 "		1.68 in.			
September		2.4 "		2.50 "		Heavy	
October		6.6 "			0.94 "		Freeze Oct. 13–14th ended season with growth immature.
November	6.7 "				Slight		
December		3.4 "	Min. 5 Deg.	Slight			Coldest month of winter.
1910							
January		0.6 "	" —2 "	1.93 in.			Precipitation mostly in form of rain.
February		0.5 "	" —10 "	2.74 "			Heavy rains the last of month.
March	10.1 "		Max. 82 "		2.15 in.		Warmest and driest March for many years.
April	3.1 "		Min. 27 "	1.00 "			High temperatures prevailed the first half of month.
May		3.7 "	" 31 "	0.84 "			Cool, cloudy and wet.

These records are taken from the Cooperative Station of the U. S. Weather Bureau at Westfield. The excess and deficiency recorded represent the departures from the normal.

this ridge is between three and four miles south of the Lake Erie shore line. From this point westward the ridge and the lake shore converge so that at Ripley the width of the fruit belt is between two and three miles. The writer examined many vineyards the day following the freeze of October 13th and it was observed that there was a gradual diminution of injury from Fredonia to Ripley while in the vicinity of the latter place no freezing temperatures had occurred. The rainfall for October and November returned to normal or a little below, and in November the temperature rose quite markedly to above normal.

December was the coldest month of the winter, the latter half being an unbroken period of cold weather with an intense cold wave occurring about the 28th. The minimum temperature for the month was 5 degrees. The precipitation was slightly above normal.

January, 1910, was slightly below the average in temperature and above in precipitation, which was mostly in the form of rain. The minimum for the month was -2 degrees.

The prevailing temperature of February was nearly normal. Several heavy snow storms occurred about the middle of the month. The minimum temperature recorded was —10 degrees. At the end of February the temperature rose rapidly and was accompanied by heavy rains.

In neither January nor February did the maximum temperature reach above 47 degrees and this only on single days.

March was the warmest and driest month for many years. The daily mean was 10.1 degrees above normal, the maximum temperature for the month was 82 degrees. In this section the precipitation was 2.15 inches below normal for the month. From March 18th to April 6th inclusive, the maximum mean was 66.1 degrees, the minimum mean was 40.3 degrees, while the mean for this period of 19 days was 53.2 degrees.

The high temperatures prevailing during the last half of March continued on into April with the rainfall for the locality about an inch above normal. On April 13th the temperature dropped to 27 degrees.

The weather of May was cool, cloudy and wet, being very unfavorable for vegetation after the climatic conditions of March and April. On May 5th the temperature fell to 31 degrees.

For the purposes of this bulletin it is unnecessary to trace further the weather as to its possible relationship to the crop of 1910, for the factors that were instrumental in the destruction of approximately 50 per ct. of the fruit buds formed during the growing season of 1909, had operated before or at the end of May, 1910.

The questions with which we are now confronted are: First, What were the conditions of the vine that favored the bud injury? Second, What were the climatic factors that actually produced the injury? Third, When were the injurious causes operative?

It has already been stated in a summary of the weather conditions of the summer of 1909, that an unseasonable freeze occurred October 13th and 14th while a considerable portion of the fruit was still on the vines At this time, while fruit had probably matured, it is very reasonable to believe that wood maturity was by no means complete. In fact, our observations during the years 1915–1916 indicate quite clearly that until an actual freeze occurs the vine extends its energies to maturation of its fruit at the expense of wood maturity; and if the unripe fruit be allowed to hang thruout the fall, wood maturity is not nearly so complete as when the fruit is picked some time previous to a freeze.

It is not contended that the minimum of 27 degrees on the 14th of October or that of 31 degrees on the 19th produced a killing of the fruit buds, but that these temperatures checked wood ripening that should normally be extended for two weeks in a season like 1909, and as a consequence the buds and canes that were to produce the crop of 1910 went into winter in a condition that would render them particularly susceptible to even normal winter temperatures.

Probably the most important determining factor in the winter injury of grape buds is maturity of the tissues of canes and buds. With the cambium and some other tissues maturity is associated with a low moisture content and thickened cell walls. However it seems probable that under unfavorable climatic conditions killing of well matured wood is possible, as will later be pointed out.

Numerous writers on winter bud injury of fruits have all recognized the relationship and importance of wood maturity with the degree of winter injury as noted in a preceding section.

With the grape, delayed maturity is clearly indicated by the succulence of the terminal nodes of the cane, with their small, newly expanded leaves. These grade gradually to the more lignified, light brown nodes, still containing considerable amounts of water, but as the base of the cane is approached, the lignification increases, the outer bark is well cutinized and the water content is lowered. These first named conditions existed during the fall of 1909 as well as in other years since.

WHEN DID THE INJURY OF 1909–1910 OCCUR?

Can we answer the query, When, during the winter of 1909–1910, did the actual killing occur? We are confronted with difficulty, as no observations were made of the dormant vines during the winter, and only at the beginning of foliation in May was it noted that many buds were failing to start. It has been previously stated that December, 1909, was the coldest month of the winter, with a minimum of 5 degrees, and that near the close of the month there was a continued severe spell of cold.

In this region there are very few days during the winter that are without strong to high winds. Bernbeck and Goeppert[29] have suggested the relationship of winds to evaporation during the dormant season and the resultant winter injury therefrom.

In late February a sudden drop in temperature to —10 degrees was recorded, but from this date to the 18th of March no unusual maximum or minimum temperatures occurred.

Beginning with March 18th the temperature rose rapidly and continued high until April 6th, a 19-day period.

Assuming that the effective initial temperatures as given by De Condolle,[30] Durand, de Gasparin, Boussingault, Lippincott and Brehm[31] are approximately correct, and the uniformity of their data indicates that they are, other conditions being equal, it is probable that enough heat units were distributed over a sufficient interval to start some activity, at least, in the dormant bud, even if not to bring the bud to actual foliation. In the observations of the workers herein quoted no mention is made of the relationship of precipitation and evaporation to the starting of bud growth. These factors no doubt are important in the foliation of plants when taken in con-

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[29] L. c. Goeppert and Bernbeck. p. 111.

[30] L. c. De Candolle, Durand, de Gasparin, Boussingault, Lippincott. pp. 113, 114.

[31] L. c. Brehm, p. 114.

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nection with temperatures. On April 13th a minimum temperature of 27 degrees occurred and a killing frost on the 12th of May.

Reviewing the data for the winter of 1909 and 1910, there are suggested three possible periods when the killing may have occurred, the first, during late December; the second, in late February and the third, subsequent to the high temperatures of March 18 to April 6. It is very probable that some injury occurred during each of these periods. Well matured wood and buds, however, would not have succumbed to the cold of December nor of February, as the minima of these months have been reached many times since 1909–1910. It is questionable whether a temperature of 27 degrees is injurious to well matured bud tissues even though they be in a swollen condition, according to Whipple.[32]

The temperatures of 1909–1910 not only killed the flower parts of the bud, but the vegetative axses as well, so that no growth developed from the injured nodes. Here is seen the extreme type of injury to fruit buds as described by Chandler.[33]

PLANT CONDITIONS RELATED TO WINTER INJURY.

GROSS MORPHOLOGY OF THE GRAPE BUD.

The grape bud is a compound one, consisting frequently of three distinct buds enclosed within the same scales. The primary or fruit producing, the secondary a wood or sterile but sometimes a fruit-bearing one, the tertiary, strictly an undeveloped wood bud, that only expands as a shoot when the others have been destroyed. The primary bud is the principal fruit producer but it may only develop a shoot; the secondary produces but small amounts of fruit at any time and this usually when the primary bud has failed, and in no event does it produce a shoot equal to the latter. With vigorous young vines the secondary bud often matures one to two clusters of fruit even when the primary bud is carrying its usual quota, but with old vines or those weakened through neglect of fertilization or cultivation, it rarely develops even a shoot if the primary bud unfolds, and is uninjured later. The primary buds on canes from wood older than two years are very frequently sterile.

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[32] L. c. Whipple, p. 111.
[33] L. c. Chandler, p. 110.

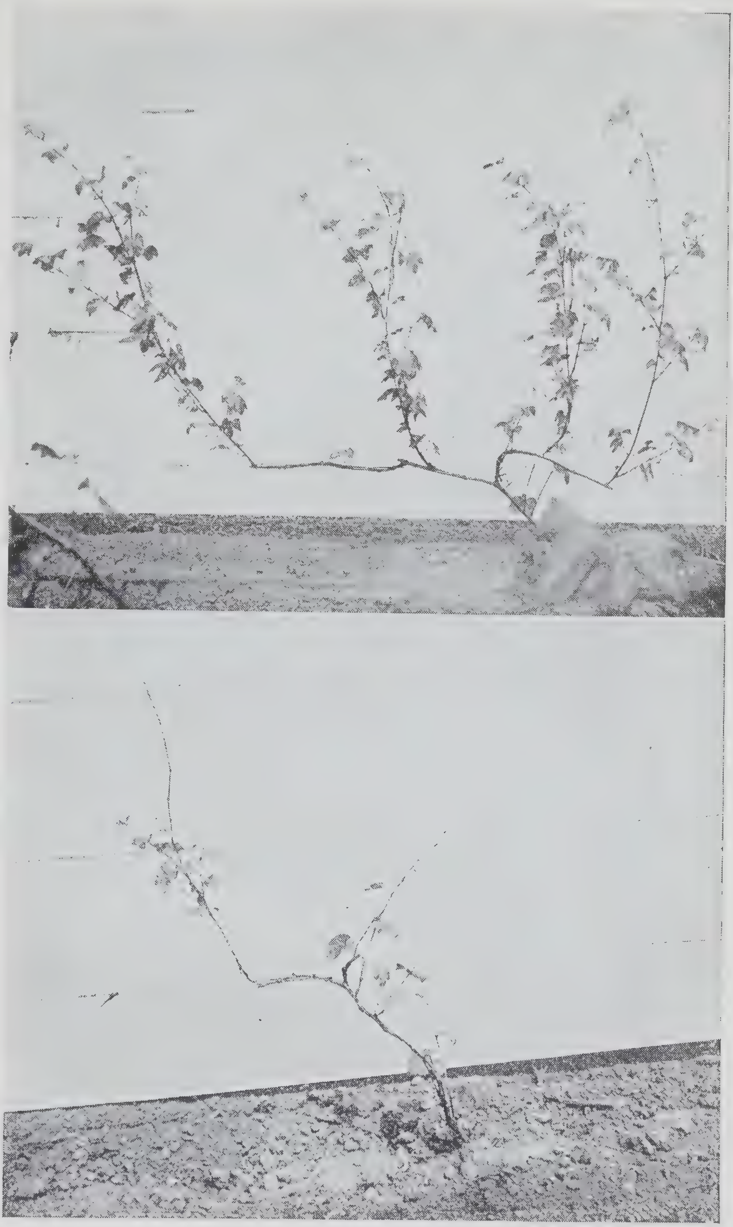

Plate LV.

Concord Vine without Growing Shoots on Some Nodes, as a Result of Winter Killing of Dormant Fruit Buds.

Lower: Moore Vine, with Injury More Complete Than with Concord.

(Vines set six years.)

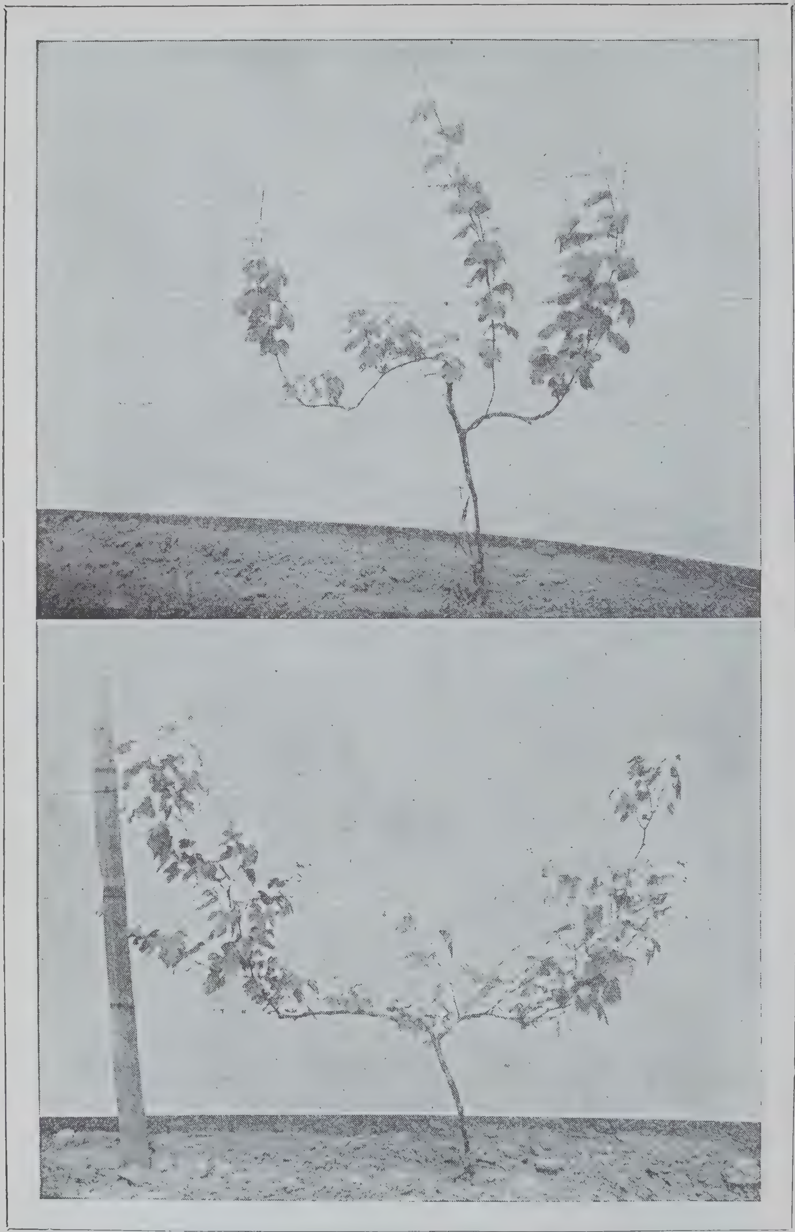

PLATE LVI.
CATAWBA VINE, WINTER-INJURED IN 1915–1916.
DELAWARE VINE IN SAME VINEYARD.
(Vines set six years.)

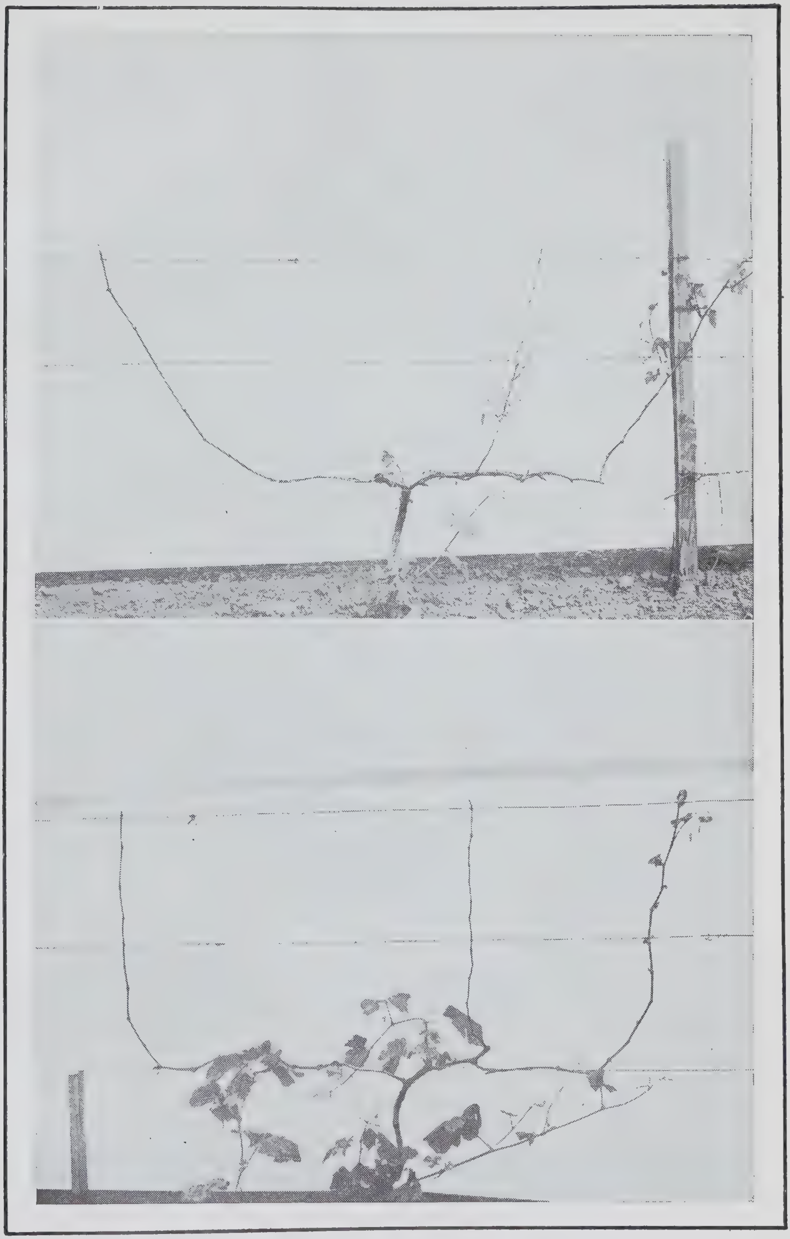

Plate LVII.

Niagara Vine Showing Severe Injury Caused during Dormant Season, 1915–1916.

Empire State Vine That Offered Little Resistance to Winter-killing, 1915–1916.

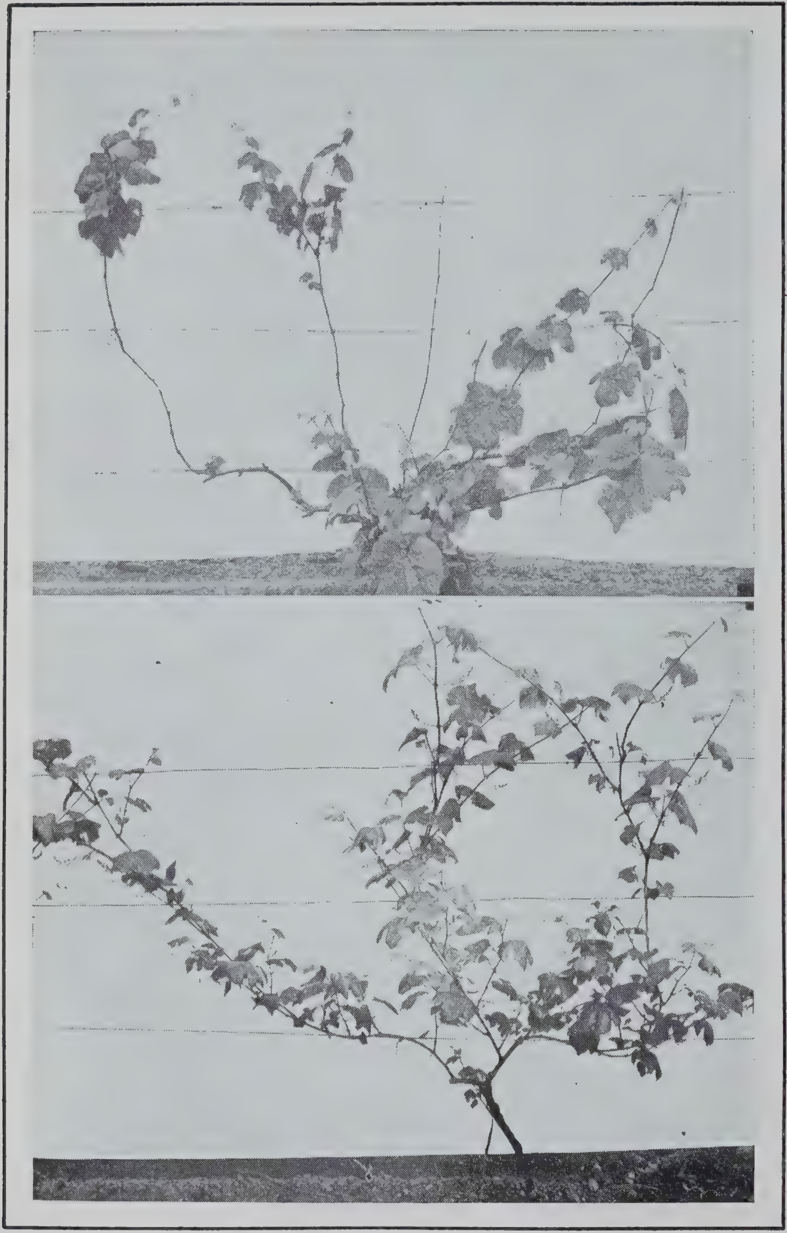

Plate LVIII.

Isabella Vine with Extensive Winter-injury. Growth from Head of Vine from Adventitious Buds on Old Wood.

Worden Vine Only Slightly Injured under Same Climatic Influences.

(Vines set six years.)

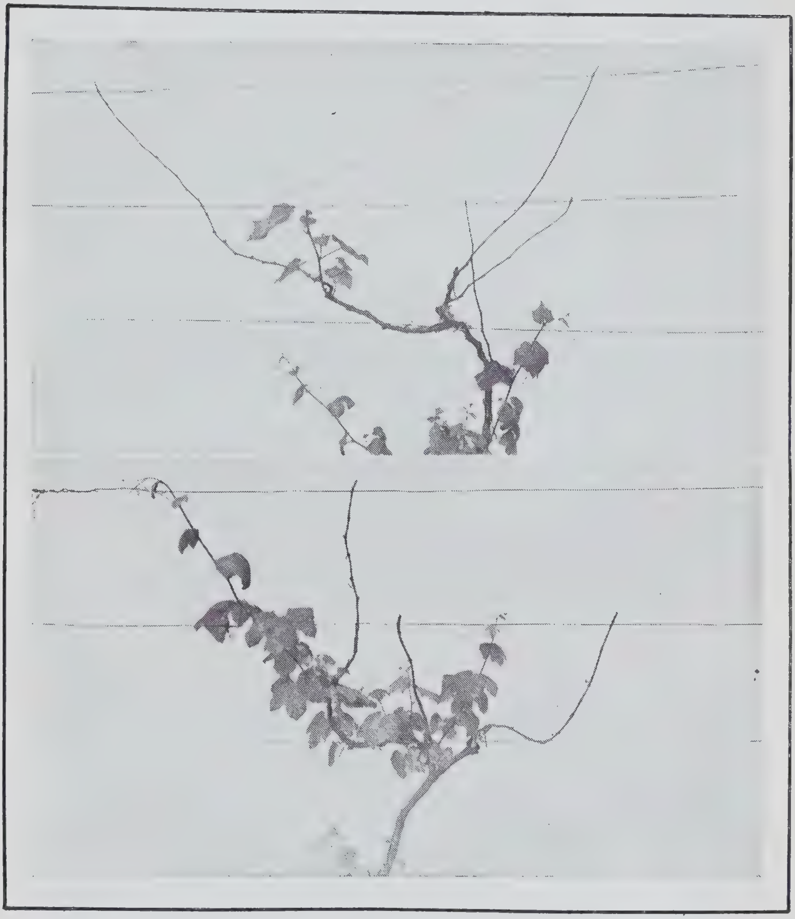

Plate LIX.

Dutchess Vine with Only One Bud on Fruiting Wood That Escaped Winter-killing, 1915–1916.

Diana Vine Located Very Near Dutchess and Almost as Badly Injured.

(Shoots on both vines largely from old wood and not true fruit-bearers.)

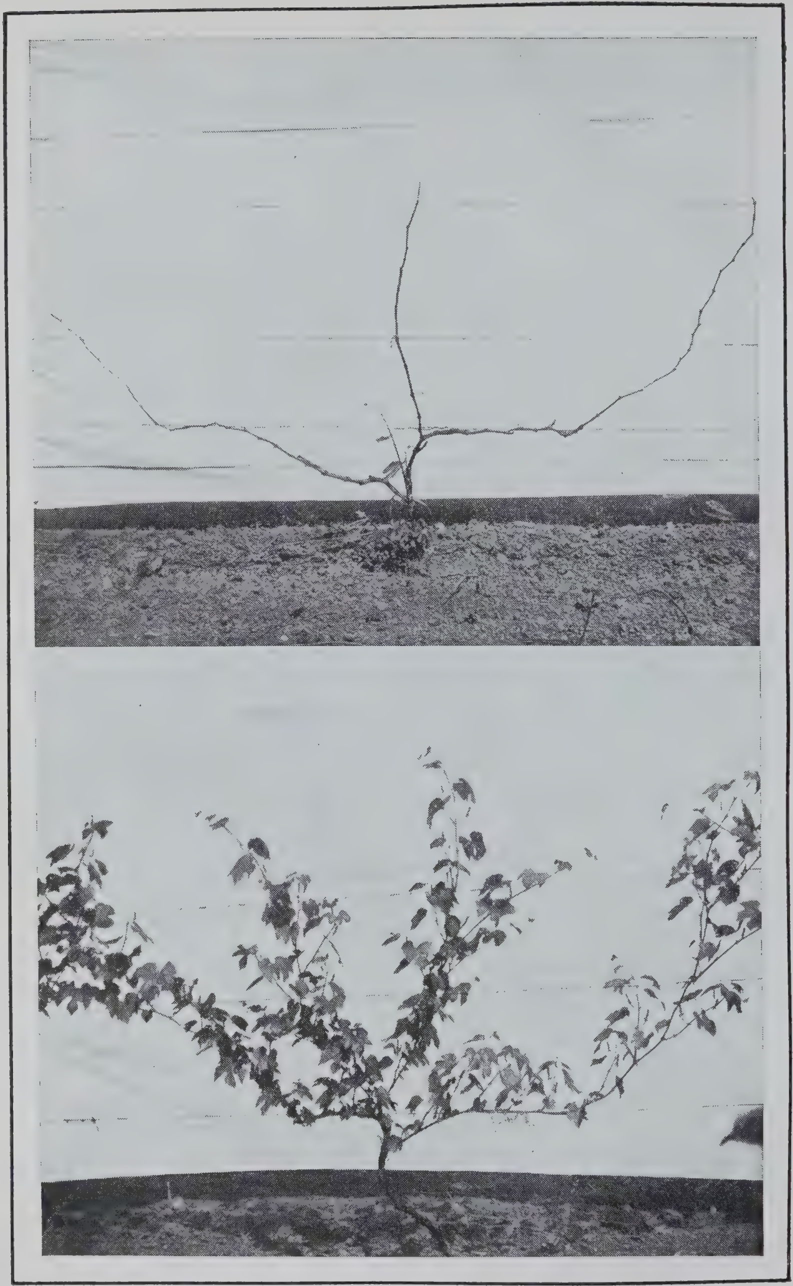

Plate LX.

Armalaga (Armlong × Malaga) Contains Much Vinifera Blood. Buds for Fruit-bearing Completely Killed.

Noah Vine Was Very Resistant to Winter-killing, 1915–1916; Only 12 Per Ct. of Buds Killed.

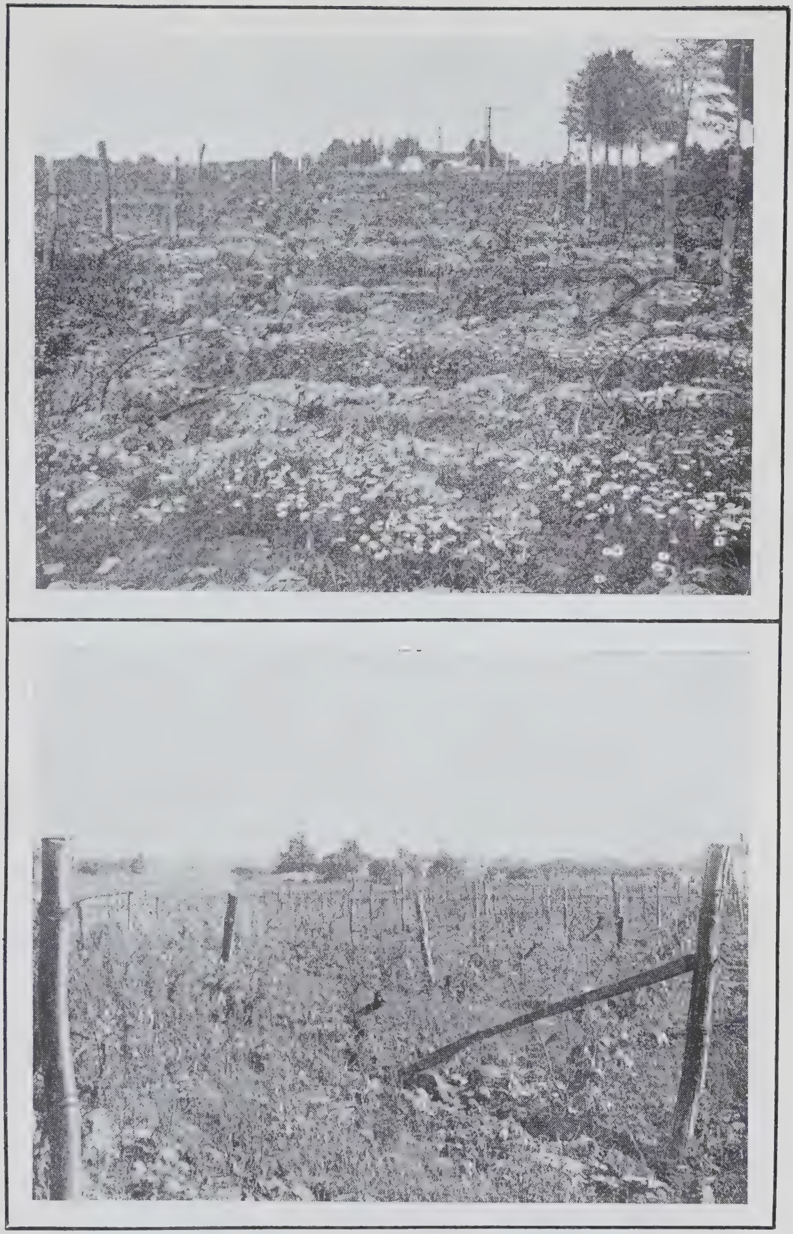

Plate LXI.

Concord Vineyard Showing Approximately 50 Per Ct. Winter-killing.

(Near Fredonia; photographed June 23.)

Concord Vineyard Showing Almost Complete Killing of Buds on One-year Wood.

(Near Irving: photographed June 27.)

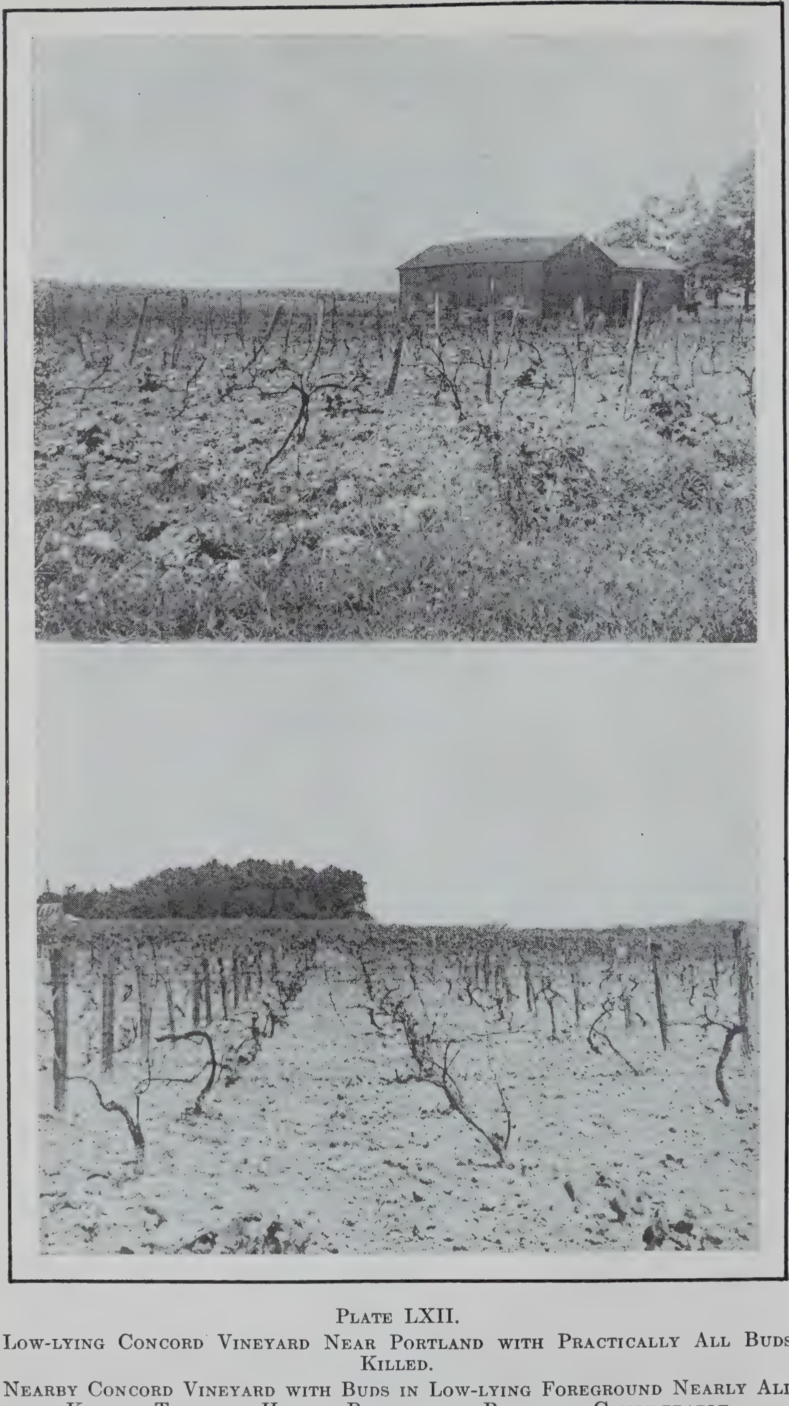

Plate LXII.

Low-lying Concord Vineyard Near Portland with Practically All Buds Killed.

Nearby Concord Vineyard with Buds in Low-lying Foreground Nearly All Killed; Those in Higher Background Produced Considerable Growth of Shoots.

MATURITY OF WOOD AND BUD AND THE SUGAR CONTENT OF THE FRUIT.

Records covering the years from 1909–1916 inclusive suggest that the sugar content of the grape in any year is quite closely correlated with cane and bud maturity of that season.

In a consideration of this statement it is interesting to note that the three lightest crops of the period, namely, 1910–1913–1916 (see tables III and IV) follow the years of the lowest sugar content, namely, 1909–1912–1915. Table III gives the sugar content of Concord for the past eight years.

TABLE III.— YIELD OF GRAPES FOR EIGHT SEASONS.

Tons per acre for							
1909	1910	1911	1912	1913	1914	1915	1916
4.5	2.15	5.52	4.32	2.4	4.7	3.22	1.88

TABLE IV.— SUGAR CONTENT OF CONCORD GRAPES FOR EIGHT SEASONS.

1909	1910	1911	1912	1913	1914	1915	1916
Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
15.83	16.15	16.87	14.17	16.76	17.51	14.56	17.56

A high sugar content does not necessarily indicate that a large crop will follow the next year, for other factors are involved. It is well known that the cane growth in a year of high yield does not equal that in the year of a light crop. If bud maturity can be approximately judged from the sugar content of the grape it will prove of great value to the vineyardist. For, knowing that buds are immature and that injury is probable during the dormant period, he can more intelligently prune, and by leaving a greater number of buds than he would ordinarily, he can produce more uniform annual crops.

WINTER KILLING AND THE OFF YEAR.

It has been suggested by Whipple[34] that winter-injured fruit buds of the apple, when the killing of the bud is not complete, may develop leaves, and that the so-called off years for some fruits are in reality such by reason of this partial injury.

Our observations with the Concord bear out this statement. In two or more seasons during the past eight years, those buds on the fruiting canes, that from their location gave every indication of normal fruit buds, failed to develop as such, but instead produced only partially or completely barren shoots. The gross morphology of the compound bud of the grape has been previously described; and taken in connection with the preceding statement it will be seen that should the more tender flower parts be destroyed by low temperatures, the vegetative part of the bud may develop a shoot. If this condition maintains in a season following a heavy crop, the presence of barren canes is usually attributed to the exhaustion of plant food through the bearing of the heavy crop. However, it seems that one plausible explanation of such a condition is that the demands of the heavy crop have withheld from some of the maturing buds the requisite amount of plant food, so that they have gone into winter poorly ripened.

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[34] L. c. Whipple, p. 111.

Thus the bearing of a heavy crop one year may affect the crop of the succeeding one. It sometimes happens that the wood and fruit will so thoroly mature even in a season of high yield that very few buds are at all injured. This is especially the case under favorable climatic conditions. The yield of 1911 in certain of the experiment vineyards at Fredonia was considerably higher than even that of 1909; while that of 1912 was approximately equal to it. The difference between the yields of 1911 and 1912 is easily attributed to a less amount of fruiting wood retained for the crop of 1912, rather than winter injury to the buds and canes.

From a comparison of Table III with Table IV it will be seen that if wood and bud maturity alone were the chief factors that determine the succeeding crop, that of 1915 would be considerably larger than the tonnage actually obtained, for the sugar content of the fruit of 1914 clearly indicated that wood maturity had been attained; however, the light yield of 1915 was largely due to a lack of well placed canes.

WEATHER CONDITIONS AND THE CROP OF 1913.

Table III shows that the yield in 1913 was on a par with that of 1910, that the yields of 1911 and 1912 were considerably above the average and from Table IV we see that the crop of 1912 ripened poorly. The differences in temperatures for either 1911 or 1913 as compared with those of 1912 are not sufficient to account for poor maturity. However, the excessive precipitation, 6 inches in September or 2.85 inches above normal, taken in conjunction with a monthly mean temperature equal to that of August, 65.4 degrees, were probably the determining factors. In pruning during the fall and winter of 1912–1913 much of the season's wood was so immature, as evidenced by the withered canes, that it was impossible to find a sufficient number coming from wood two years old; and so in order to keep the fruiting wood close to the head it was necessary to prune to canes growing from wood older than two years. Buds on canes from the latter are not the best fruit buds, but rather produce wood. Our records show that while 1477 pounds of wood per acre was pruned away in the fall and winter of 1911–1912, but 1098 pounds was pruned in the fall and winter of 1912–1913 and less buds per vine were retained than in 1912.

Thus the probable 1913 crop was largely discernible at the fall and winter pruning of 1912–1913, when the immature canes were cut away, altho many buds that were retained at the time afterward succumbed to low temperatures during the winter. But missing buds were not nearly so apparent at foliation as was the case in the early summer of 1910.

The vineyardist generally ascribes the light yield of 1913 to a lack of wood growth. This is in part true, but as has already been stated the real lack of fruiting wood was essentially a lack of well matured wood that was resistant to low temperatures. With well matured wood in 1912 it is reasonable to believe that the yield of 1913 would not have dropped to the low stage of that year, as the extremes of the winter temperatures that followed were not great nor was a lower minimum than zero reached during the period.

The information for 1912–1913 is brought forward in this bulletin merely to show the relationship of immature wood and buds to the succeeding crop. The data we have taken for the year is not a true indication of the amount of injury that would have occurred were the pruning delayed until late spring, so that the poorly matured canes could have been observed at foliation.

WEATHER AND CROP OF 1915–1916.

RELATION OF CLIMATIC CONDITIONS TO WOOD MATURITY AND SUBSEQUENT INJURY.

The season of 1915–1916 gave abundant opportunity to study bud injury as affected by immaturity and low temperatures. These studies include not only work with the principal variety (Concord) of the Chautauqua area, but a large number of named varieties.

TABLE V.—SUMMARY OF METEOROLOGICAL DATA, WITH SPECIAL PHENOMENA AFFECTING THE GRAPE CROPS OF 1915 AND 1916 AS COMPARED WITH 1914.

MONTH.	TEMPERATURE.			PRECIPITATION.			Remarks.
	RANGE.			RANGE.			
	Daily excess over 1914.	Daily deficiency below 1914.	Extremes.	Monthly excess over 1914.	Monthly deficiency below 1914.	Extremes.	
1915							
May		5.2 Deg.			2.24 in.		Frosts of 15th and 27th killed grape foliage in places.
June		1.3 "		1.65 in.		1.28 in. in 24 hrs.	
July		2.5 "		6.11 "		1.60 in. in 24 hrs.	
August		4.6 "		0.23 "		1.04 in. in 24 hrs.	
September	3.2 Deg.			1.10 "			
October		1.3 "		0.46 "		1.12 in. in 24 hrs.	
November	1.6 "		Max. 71 Deg.	0.99 "			But little snow.
December	1.4 "		Min. 13 "	0.65 "			Snow covered ground entire month. A total of 27.3 in.
1916.							
January	4.8 "		Min. 1 "		0.61 "		Sudden changes. Many gales. No snow. Extreme warm period.
February	1.8 "		Min.—13 "	0.82 "			Several days below zero. A daily range of 47 deg.
March		6.2 "	Min.—16 "		0.56 "		Great temperature extremes. A severe glaze storm. Snow.
April	0.8 "		Min. 25 "	0.53 "			Max. for month 75 deg.

These records were kept at the Vineyard Laboratory, Fredonia, N. Y., cooperating with the U. S. Weather Bureau.

It will be necessary, for an understanding of the effects that followed, that the climatic conditions of the growing and maturing periods of 1915 be studied, as the unfavorable agencies were operative during those periods.

Table V summarizes the meteorological data, with special phenomena affecting the grape crops of 1915 and 1916 as compared with 1914. The season of 1914 is selected for comparison as the yield of that year was considerably above the average, and the fruit ripened well, as is shown by the sugar content in Table IV.

The wood also matured well, for not enough injured buds were noted in the spring of 1915 to warrant taking any data.

The records given in Table V show that for the growing months of May, June and July there was a total deficiency of 277 degrees, or in other words for each day of this period the temperature was 3 degrees below that of 1914. For the maturing months of August, September, and October the total deficiency was but 87 degrees. But September gave a daily excess of 3.2 degrees or a total of 96 degrees for the month. During August, a critical month in the ripening of the fruit, the daily deficiency was 4.6 degrees or a total for the month of 142 degrees.

The total precipitation for the growing months of May, June and July was 5.52 inches above that of 1914, while for the maturing period it was 2.8 inches above that of 1914, August showing an excess of .23 inches, September 1.10 inches, and October about one-half an inch. The total rainfall, however, does not tell the whole story, for the frequency must also be considered in a correlation of weather with the growing and maturing crop. Table VI, on the page opposite, illustrates the distribution of the rainfall and the condition of the sky for the season of 1915.

From this table it will be seen that some rain fell on approximately one-half of the days of the summer and that of the 183 days included from May to October, 65 were classed as cloudy, while 59 were partly cloudy. These figures are essentially correct, there being very little variation in either direction.

The cloudy days were about equally distributed thru the two periods, the growing and maturing, altho the greatest excess precipitation, 6.11 inches, of any month, occurred during July. The cloudy condition of the sky as well as the large number of rainy days during August, September and October retarded evaporation so that the soil was kept wet. This condition, coupled with the temperatures of September that were above normal, kept wood growth active late, and retarded the ripening of the fruit. In fact, strictly speaking, the fruit did not mature, as is indicated by the sugar analysis of 1915 shown in Table IV.

TABLE VI.— DISTRIBUTION OF RAINFALL AND THE CONDITION OF THE SKY DURING 1915.

	Rainy days.	Cloudy days.	Partly cloudy days.
	No.	*No.*	*No.*
May	15	16	6
June	14	6	10
July	18	10	14
August	18	14	5
September	13	8	12
October	15	11	12

The immaturity of wood during the fall and winter of 1915–1916 was not apparent as in 1912–1913. In the vineyards under consideration practically the same number of canes per acre were put up in 1916 as in 1915, while the amount of wood pruned away was not much less than that removed in pruning for the 1915 crop. In short, enough fruiting buds were retained for the 1916 crop, that normally would produce a tonnage equal to that of 1915. There was no indication whatever during either fall or winter of poorly matured wood. In fact not until early spring, before foliation, was it determined that a large percentage of canes and buds were injured or dead.

What were the weather conditions of the winter season of 1915–1916 that brought about the extensive injury and killing? Table V summarizes these.

According to our weather records the November temperatures were above normal as well as those of 1914. There was no snow the first half of the month, and then only a trace till the latter part. During the last two days about four inches fell. The maximum reading for the month was 71 degrees while the minimum reached 24 degrees.

During December the temperatures were fairly uniform, with the greatest range on any day reaching 26 degrees. The ground was covered with snow thruout the month, while a total of 27.3 inches fell. A glaze storm occurred on the 13th. The maximum for the month was 50 degrees with a minimum of 13 degrees.

January was remarkable for sudden temperature changes, many days of rain and very high winds, gales occurring on the 10th, 13th, 16th and 18th. The ground was bare nearly the whole month, there being only two days when there was 5 inches or less of snow. During the week January 22–29 for 96 consecutive hours the average hourly temperature was 52.6 degrees. The maximum for the month was 69 degrees while the minimum was 1 degree.

February was somewhat below the average in temperature, when for several days the readings were below zero. A range of 47 degrees was recorded in one day. Some snow fell on 19 days during the month, but by the middle there was 5 inches or less on the ground. At the end of the month 10 inches of snow covered the ground. The maximum temperature recorded was 47 degrees, the minimum —13 degrees.

March was a month of great temperature extremes with the mean temperature of the month below normal. Snow covered the ground till near the end. A severe glaze storm occurred on the 6th and 7th. The maximum for the month was 58 degrees while the lowest minimum of the winter —16 degrees was recorded.

The monthly mean temperature for April was slightly below the normal. The maximum reading for the month was 75 degrees while the minimum was 25 degrees, the last below freezing temperature of the year. The total precipitation for April was 2.19 inches above normal, this largely in the form of rain. However April or subsequent climatic conditions were not factors in a determination of the injury of this season, for as has already been stated, it was noted during the latter part of March that a large percentage of buds were entirely dead, while others showed only partial injury. Many buds at foliation, apparently uninjured, partially or entirely expanded only to die back later. Still others expanded as leaf buds only, the flower parts being killed. Some of the vegetative shoots grew several inches and then withered.

APPEARANCE OF THE INJURED AREAS.

An examination of many canes on which injured or dead buds and shoots were noted, invariably showed blackened areas along the cambium layer, while the tissues of the canes were surcharged with water. The cut surfaces of the canes lacked the vivid green of the normal tissues, but rather were of a grayish tinge. The areas around the juncture of the buds with the tissues of the canes contrasted strongly as to the depth of color with those uninjured. The cut surfaces of the former instead of showing the normal vivid green were of a yellowish brown color.

IMMATURITY THE DETERMINER OF THE INJURY.

Whatever other conditions contributed to the winter killing of 1915–1916, the immaturity of the cane and bud tissues is the factor that determined the extent of the injury. The poorly matured wood was conditioned upon the climate of the previous growing and maturing periods. Local factors such as late cultivation, elevation, soil type and the moisture content thereof no doubt aggravated or lessened the extent of the injury.

SPRING FROSTS AND THEIR RELATION TO WOOD MATURITY.

The frost injury of May, 1915, that affected many vineyards, especially in the eastern end of the " Belt " was considered as solely influencing the subsequent killing of 1915–1916, but vineyards unharmed by the May frost suffered almost as severely as those that were severely frozen back in the spring. However examination of several vineyards that were so injured during May, lead to the conclusion that such as were pruned back severely, just after the freeze, suffered considerably more from winter killing, during the dormant season 1915–1916 than those unpruned. Here again the injury may be charged to immaturity, as the closely pruned vines developed but a few canes, which grew very rank.

UNSEASONABLE WINTER TEMPERATURES AND THE INJURY.

Just how much the unseasonable temperatures of the week of January 22–29 contributed to the killing is problematical. It is worthy of note that for 96 consecutive hours of this week, the average hourly temperature was 52.6 degrees. Brehm[35] shows that the

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[35] Brehm. *Horticulturist* 18: 52–53.

Delaware comes to leafing after a mean daily temperature for 14 days of 52.41 degrees. In the Station vineyard, Delaware began leafing one day later than Concord in 1916, hence the effective initial temperatures must approximate the same. In view of these facts it is quite probable that the effective initial temperatures of January 22–29 holding for one-fourth of the time necessary to bring into leaf did start into active growth the tissues of cane and bud. Their immature condition rendered them much more responsive to high temperatures than they would have been had complete maturity been reached during the previous fall. The minimum temperature (—16 degrees) of the winter 1916 occurred in March, but if the tissues were no longer completely dormant it is possible that the earlier temperature of —13 in February might have been the fatal one.

Warm winter weather, which is not sufficient to affect the temperature of the soil, stimulates a flow of sap in the vine as well as in certain trees.

The starting of dormant buds into growth is due to the warmth they receive and is practically independent of root action. However Chandler[36] found that the scales of peach buds do not serve to protect them from low temperatures. The scales however may serve to protect the bud tissues from loss of water by evaporation.

In view of this conclusion the actual injury to grape buds during 1915–1916, if it occurred immediately subsequent to the warm period of January, was probably due to excessive evaporation rather than the formation of ice crystals within the inter-cellular spaces of the tissue, with the resultant disorganization of the cell protoplasm.

The writer did not examine the buds at the close of the abnormally warm period of January 22–29, hence it is not known how greatly they had swollen and how much the bud scales had separated.

RELATION OF NITROGEN, PHOSPHORUS AND POTASSIUM TO WOOD AND BUD MATURITY.

The experiment vineyards afforded abundant opportunity during the spring and summer of 1910 and 1916 to study any possible relationship between the kind of fertilizer treatment with bud injury. With certain crops potassium has been ascribed as hastening maturity, while with others it shows the opposite effect. Nitrogen it is known delays maturity, while phosphoric acid on the other hand has been demonstrated as bringing some crops to early maturity, especially during a wet season.

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[36] L. c. p. 110.

The writer is unaware of any available data relating to these functions with tree fruits and more particularly the vine.

In the spring of 1910 as soon as it was seen that many buds had failed to start, an inquiry arose as to the possible effect of fertilizers upon wood maturity and winter killing of grape buds. Our counts were made in a section of Concord vineyard about 30 years planted, situated upon a loose gravelly-loam soil of great depth. This is naturally well drained and in time of drouth the vines frequently are affected by a lack of moisture.

TABLE VII.— COMPARISON OF FERTILIZER TREATMENT WITH THE EXTENT OF INJURY FOR THE YEAR 1915-'16.

PLATS.	Total buds.	Dead buds.	Amount of injury.
			Per ct.
Complete fertilizer and lime	495	154	.311
Complete fertilizer	607	151	.248
Nitrogen and phosphorus	491	94	.191
Nitrogen and potash	632	165	.261
Phosphorus and potash	495	117	.236
Check	453	110	.242
Complete fertilizer and lime	699	165	.236
Complete fertilizer	647	155	.239
Nitrogen and phosphorus	610	153	.250
Nitrogen and potash	570	142	.249
Phosphorus and potash	553	132	.238

The 3-acre area slopes slightly to the south with the direction of the rows. From east to west the topography is very uniform. In 1909 the crop on this vineyard averaged 4.5 tons per acre. The section is divided into 11 plats that in the spring had received various combinations of nitrate of soda, dried blood, muriate of potash, acid phosphate and lime.

RANGE OF INJURY IN 1909–1910.

The counts made in the spring of 1910 showed that the extent of injury did not vary beyond the error liable in making the count, between the various fertilized plats and the control rows. Each plat indicated that about 50 per ct. of the buds were dead, and suggested that nitrogen, phosphorus, potassium or calcium had not influenced favorably or unfavorably wood and bud maturity. It may be contended that enough time had not elapsed between the applications and the maturing period to make the influence of the fertilizers felt.

Table VII presents a comparison of fertilizer treatment with the degree of injury for the year 1915–1916 in the same vineyard.

The counts from which the percentages were figured include all the fruiting buds reserved for producing the crop of 1916, with the exception of short spurs on wood older than two years that were reserved for a renewal of the vine framework. The fruit buds were borne on canes developed during the summer of 1915.

An examination of this table indicates that the injury ranged from 19 to 31 per ct. The differences between the variously treated plats are so slight, that they come well within the range of experimental error, and we thus conclude that no plant food element has indirectly influenced the injury in this vineyard.

Tables VIII and IX present similar counts made in the same season in two seven-year Concord vineyards situated on Dunkirk silt loam. The area of these is 3 and 4 acres respectively. The entire area was tile-drained before the vineyards were planted.

TABLE VIII.— WINTER INJURY ON THE PLATS OF SECTION 8, 1916.

PLATS	Total buds.	Dead buds.	Amount of injury.
			Per ct.
Complete fertilizer and lime	487	192	.394
Complete fertilizer	489	179	.366
Stable manure	(Tile) 470	146	.310
Check	384	93	.242
Nitrogen and potash	456	132	.289
Nitrogen and phosphorus	451	176	.390
Stable manure and lime	(Tile) 528	153	.289
Check	480	120	.250
Clover	499	153	.306
Clover and lime	511	152	.297
Phosphorus and potash	489	134	.273
Check	(Tile) 433	121	.279
Barley and turnips	433	128	.295
Barley and turnips, limed	406	103	.253

TABLE IX.— WINTER INJURY ON THE PLATS OF SECTION 9, 1916.

PLATS	Total buds.	Dead buds.	Amount of injury.
			Per ct.
Complete fertilizer and lime	553	157	.283
Stable manure	501	153	.305
Check	(Tile) 430	144	.334
Complete fertilizer	542	172	.317
Stable manure and lime	517	162	.313
Nitrogen and phosphorus	619	200	.323
Nitrogen and potash	575	193	.335
Check	(Tile) 595	219	.368
Phosphorus and potash	523	156	.298
Clover	440	172	.391
Clover and lime	427	163	.381
Check	(Tile) 390	178	.456
Barley and turnips	475	180	.378
Barley and turnips, limed	443	184	.415

Where the word tile occurs it is indicated that a run is located in the center of the plat and parallel with the direction of the rows.

In vineyard 8 the range of injury varied from 25 per ct. to 39 per ct., while in vineyard 9 the range was from 28 to 41 per ct. In neither were the differences among the plats sufficient to warrant any definite conclusions as to the effects of fertilizers upon wood and bud maturity, and indirectly upon the degree of winter injury. Further the plats thru which the tile ran were apparently no better favored than those plats lying at the farthest point from the run. This clearly indicated one of two conclusions, either the entire area was sufficiently well drained, regardless of distance from the tile, or else the amount of water in the soil during the fall was evenly distributed over the plats. The former appears the more reasonable for in no case is a row of vines further away than 20 feet from the tile, while the average distance is considerably less. It is very probable that the draw of the tile in this soil type is considerably greater than the actual maximum distance here existing between the runs. The appearance of the entire area during the wet periods of the year indicates general good drainage.

OBSERVATIONS MADE IN OTHER VINEYARDS IN 1916.

Many vineyards under various exposures and locations were examined during the summer of 1916 and notes of their condition taken. Descriptions of a few of these follow:

Vineyard F. was examined on June 23 and photographs of parts of the area made. The injury in this vineyard probably averaged from 50 to 60 per ct. of dead buds. It is wet over a large part but especially along the main highway where the water is backed up by the road. During spring and late fall it frequently stands over this portion in large ponds. It is over this area that the greatest injury was shown.

Vineyard W. showed very little winter killing of the buds, probably less than 5 per ct. The only injured canes were in a low corner that is very wet. The larger part of the vineyard lies high. A neighboring vineyard, similarly situated, likewise escaped serious injury, but another on the same farm, laying between two woodlots and situated very low, was almost completely killed back. Practically all vineyards in this locality but the two first named were so badly injured that only very small crops were harvested. The greatest injury in all cases was on the low wet areas.

Examined vineyard L. June 27th. All the sections on this farm are severely injured; however the upper end of one section that is situated high, escaped. All other sections are very wet and on most of these the crop will not pay for the handling.

Vineyard G.— Catawba was examined June 29th and this variety was found badly injured. It is low lying and on a heavy soil. At this date it indicates that it has been very wet. The owner estimated his crop at 5 per ct. of the ten-year average.

There were but very few vineyards that escaped some injury, while taking the " Belt " as a whole the crop records show that the yield of 1916 was but 60 per ct. of the 1915 return or 71 per ct. of the seventeen-year average.

VARIETAL (OR SPECIES?) RESISTANCE TO LOW TEMPERATURES.

Table X shows the extent of winter killing of 146 varieties of grapes during the winter of 1915–1916. These are practically all of the same age, in the same section, under similar soil conditions,

TABLE X.— PERCENTAGE OF WINTER INJURY TO GRAPE BUDS OF DIFFERENT VARIETIES FOR THE WINTER OF 1915–1916.

(100 per ct. indicates that every bud reserved for fruiting was killed.)

	Per ct.
Agawam	59
America	26
Aroma	15
Aug. Giant	73
Bacchus	40
Bailey	30
Banner	55
Barry	17
Beacon	51
Berckman	42
Brighton	82
Brilliant	67
Brown	62
Campbell	34
Carman	28
Catawba	56
Champion	64
Clinton	41
Colerain	32
Columbian	41
Cottage	13
Cynthiana	52
Delakins	94
Delaware	56
Diamond	24
Diana	86
Dracut Amber	15
Dry Hill	48
Dutchess	94
Daisy	29
Ohio	49
Moore	52
Victor	29
Esther	22
Eclipse	65
Clevener	44
Elvira	25
Empire State	80
Etta	26
Eumelan	76
Gaertner	57
Geneva	63
Goethe	60
Green Mt	49
Green Ea	83
Hartford	70
Hayes	48
Headlight	34
Herbemont	100
Herbert	30
Hernito	54
Hicks	27
Iona	78
Isabella	95
Ives	31
Jefferson	78
Jessica	27
King Philip	70
King	76
Lady	46
Lady Washington	99
Lindley	49
Little Wonder	16
Lucile	21
Lukfata	70
Lutie	26
Martha	20
McKinley	74
Caco	35
Manito	26
Mo. Reisling	27
Massassoit	26
Mericadel	34
Merrimac	74
Mills	100
Montefiore	43
Moore Diamond	14
Moyer	36
Nectar	57
Niagara	64
Noah	12
Norton	54
Northern Muscadine	22
Perkins	36
Pocklington	19
Reaqua	29
Regal	46
Rockwood	27
R. W. Munson	22
Salem	50
Steuk	39
Armalaga	99
Telegraph	33
Triumph	77
Ulster	92
Vergennes	68
Wilder	36
Wine King	22
Woodruff	15
Worden	24
Wyoming	78
Red Reisling	31
Blue Black	57
Black Pearl	21
Marsala	50
Neosho	24
Beta	19
Hungarian	56
Geant	65
Starks Delicious	64
Marion	28
Emerald	54
Secretary	88
Gold Coin	34
Eumadel	92
Bell	24
Hubbard	14
Delago	40
Kentucky	65
Concord	18
Gloire	29
St George	29
101	28
3306	67
Captivator	96
Canada	74
Ozark	30
Black Eagle	100
Rebecca	100
Butler No. 1	66
Ripley (Station 98)	31
Dewdrop	80
Ontario (Station 95)	82
Station 939	90
Urbana (Station 3518)	77
Westfield (Station 3516)	99
Grand Feuilles	21
264	20
265	38
Constantia	39
Brocton (Station 3345)	48
268	100
267	100
Solonis	60
Dog Ridge	90
Mission	10
Salt Creek	62
Grand Glabre	70
Vitis Berlandieri	90

a gravelly loam, with the exposure uniform. All have been subjected to the same cultural practices. The basis for the computations was the same as for the Concord, namely, all buds developed during 1915, which were normally the fruit buds of 1916, were counted.

Of the native varieties it is seen that the injury ranged from 12 per ct. to 100 per ct. or complete killing of the fruit buds. Mission, a variety of the species Rupestris, however, was the least injured with 10 per ct. of its buds killed.

This record is particularly interesting in one respect. It is popular opinion at least, that the carrying of a crop, or a heavy one in particular, delays maturity regardless of other factors. Riparia, Gloire, Rupestris St. George, No. 265, No. 268, Mission and Grand Feuilles are all male plants and consequently set no fruit. In other years they have shown no greater nor less winter killing than has Concord, yet only one, Mission, was less affected than Concord in this vineyard during 1915–1916. Nos. 264 and 265 are female and male plants respectively of the same species, *Vitis riparia.* Since practically no fruit was borne on No. 265 during 1915 no fair comparison of the degree of injury between the sexes is yet possible. Nos. 267–268 are female and male plants respectively of the same species, *Vitis champini.* In this instance no crop was matured by 267 in 1915.

However, while it cannot be conclusively stated, yet it is apparent that too much stress has been given to the relation of wood and bud maturity to the size of the crop irrespective of other factors, principally climatic.

To what is the difference of susceptibility between two varieties due? Is it an inherent specific character? Assigning the varieties included in Table X to the species from which they have been derived, it is apparent that there are wide differences of susceptibility, even among varieties derived from the same species. Of the pure Labrusca varieties, the range of killing is from Cottage with 13 per ct. of injury to Wyoming with 78 per ct. The varieties containing Riparia blood along with that of other species, vary in degree of injury from the minimum of 12 per cent with Noah, to 88 per ct. with Secretary. Varieties derived in part from Aestivalis range from 22 per ct. injury with Wine King, to 94 per ct. for Dutchess. This group as a whole however shows a percentage of injury slightly greater than the Riparia varieties, and considerably greater than the Labruscas. Injury with the Labrusca-Vinifera hybrids varies from 15 per ct. for Woodruff, to 100 per ct. with Mills and Rebecca. The percentage of injury for all varieties of this group is but slightly less than for the Aestavalis. However the varieties of the Labrusca-Vinifera group in which the Vinifera fruit characters are more prominent, as a rule show a higher percentage of injury than those in which the Labrusca characters predominate, and also a greater percentage than those with Aestavalis blood. It has been thoroly proven that pure Vinifera varieties practically kill back to the ground in New York during winter if left uncovered.

Pure Riparia represented by Gloire and Grand Feuilles with 29 per ct. and 21 per ct. injury respectively show a consistent, fairly high resistance. With the pure Rupestris varieties, St. George and Mission, the variation between the two is greater than with the two pure Riparia. Mission, of all the varieties recorded, was the least injured with 10 per ct. of buds killed.

The resistance to injury for varieties derived from the same two or more species as the Labrusca-Vinifera hybrids is probably due to the degree of dilution of the hardier Labrusca in certain sorts than others. The extent of dilution not always being apparent in the fruit, the variety is assumed to have inherited other characters of both parents in equal degree.

However, some, as Barry for example, show more clearly the lack of Vinifera fruit characters than others from the same cross, as for example Goethe and Gaertner.

It would seem in view of these data that certain species are more resistant to low winter temperatures than others, or that wood and bud maturity is reached earlier and more completely in some species than in others.

Beach and Allen [37] found that the hardness of the wood of certain apple varieties was quite closely correlated with hardiness or resistance to low temperatures.

It is well known that the texture of wood among grape species varies considerably. However there have been no investigations, to our knowledge, that have considered the possible correlation of wood hardness with hardiness to cold.

These data indicate that there is no complete immunity from low temperatures among species or varieties if immaturity is not complete at the beginning of winter. The nearest approach was Mission with a 10 per ct. killing.

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[37] L. c. Beach and Allen, p. 12.

CONCLUSIONS.

The question now arises, Is it possible to influence the maturity of grape tissues? If so to what extent and how? With the unfavorable season of 1915 little could have been done that would have entirely eliminated the injury, yet we are convinced that the injury could have been considerably lessened in many instances. The experiences of some growers in severely cutting, in the spring, frost-injured vineyards, thus inducing a rank growth of wood, indicates that this is a doubtful practice, but rather it is better that all uninjured growth no matter where located on the vine, be allowed to develop. Excess water in the tissues being correlated with immaturity during late summer and early fall, it is obvious that practices tending to reduce the available supply are desirable. It is the opinion of the writer based on many observations during the past few years, that poor drainage has determined the injury in the majority of cases. Many vineyards are growing on soils that should never have been planted to grapes, at least until underdrainage had been installed. Such as these will never be immune to low temperature influences. It is possibly true that many such soils will return as much profit per acre in vineyards as could be obtained from general farm crops, yet there is always the prospect of heavy losses from low temperatures.

It is the customary practice to discontinue all vineyard cultivation in late July or early August. The time however should be conditioned upon the character of the weather, especially as to the rainfall. After discontinuance, all weeds should be allowed to grow, and it is a doubtful practice to mow these just before the beginning of harvesting, as is quite generally done. The mulch provided by the mown weeds at this period conserves soil moisture, a thing that should be avoided. Green manure crops operate in converse ways in regulating water in the soil. While the plants are but partially grown, all kinds no doubt produce a drying effect upon the soil, but those possessing broad leaves as rape and in lesser degree cow-horn-turnips later exert a shading, and especially where rainfall is abundant, results in a retention of more moisture than is withdrawn thru the tissues. This in a large measure may be corrected by a much thinner seeding than is ordinarily practiced. With rye, wheat, oats, barley, buckwheat, etc., the shading effect is at a minimum. Slowly available forms of nitrogen should be avoided unless applied very early. Under this restriction come such as raw bone, leather scrap, hair, etc.; especially is this true if no green manure crop be used. Excessive or late applications of nitrogen in any form favors immaturity.

The strictest application of these principles however will not suffice to entirely prevent winter injury from low temperatures, but if the injury is conditioned upon immaturity of bud and cane tissues, adherence to the practices above outlined will temper considerably the degree of killing.

CULTURE OF THE GLOBE ARTICHOKE.*

J. W. WELLINGTON.

SUMMARY.

The Globe artichoke has been successfully cultivated on the Station grounds for several years.

Altho generally known as *Cynara scolymus* Linn., this vegetable is really a variation of cardoon, *Cynara cardunculus* Linn.

The horticultural varieties are not clearly defined as is shown by the differences between seedlings of a named sort.

The plant is not fully hardy in our latitude and requires covering to endure the winter. Coal ashes proved to be a satisfactory material with which to cover the artichoke, affording sufficient protection without causing decay of the crowns.

From records taken, it is evident that marked variations exist in the producing capacity of individual plants of a variety.

Consequently the separation of the offshoots from the old main root of the plant is the most reliable method of propagation and should materially assist in the establishment of uniform and meritorious strains.

The edible portion of the artichoke is the flower bud.

The plant itself is remarkably thrifty in growth and is practically free from fungus and insect pests. One insect, a black aphid or louse, becomes troublesome at times but is satisfactorily controlled by spraying with a properly prepared dilution of Black Leaf 40 and whale-oil soap.

GENERAL INFORMATION.

The Globe artichoke (*Cynara cardunculus* Linn.) is a perennial plant which under favorable environment may produce profitable crops for several years. It has been successfully grown on the Station grounds for several seasons. Special attention has been given to winter protection and to a study of variations within a horticultural variety. The Globe artichoke, altho widely distributed in this country, has never become a general favorite among vegetables because of its varying ability to survive our Northern winters and also because of the prejudice against uncommon foods.

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* Reprint of Bulletin No. 435, May, 1917.

BOTANICAL CLASSIFICATION.

Index Kewensis[1] classifies the Globe artichoke as *Cynara cardunculus* Linn., making it a variation of cardoon and not a distinct species. The commonly used and accepted species designation, *Cynara scolymus* Linn., is given as a synonym. From observation of the two vegetables as they grew on the Station grounds this past season one readily accepts the supposition that the two are closely related — cardoon with spiny leaves and flower buds seeming to be but crude forms of Globe artichoke. At a short distance the two cannot be distinguished. Jerusalem artichoke, *Helianthus tuberosus* Linn., is a distinct species belonging to another genus of the Compositæ family. In the Jerusalem artichoke the edible portion consists of the underground tubers. Because of their common name — artichoke — the two vegetables are often confused in the minds of those who know only one or neither of the two species.

HISTORY.

De Candolle[2] believed the artichoke to be derived from the cardoon and, since the latter is indigenous to the sandy shores of the Mediterranean Sea, there must also be the home of the artichoke. Sturtevant[3] came to the conclusion that the artichoke was not utilized as food until the fifteenth century. It is asserted that the artichoke was first introduced into England in 1548.

VARIETIES.

The horticultural variations of the Globe artichoke are not sharply defined, due undoubtedly to the ease with which the flowers are naturally crossed. Hence seedlings do not always come true to type. Vilmorin[4] mentions thirteen varieties differing in shape and color of the flower buds and in the presence or absence of spines.

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[1] Hooker & Jackson. *Index Kewensis* 1:686. 1895.
[2] De Candolle. *Orig. Cult. Pls.*, p. 93. 1885.
[3] Sturtevant, E. Lewis. *Am. Naturalist*, 21:126. 1887.
[4] Vilmorin. *Les Pls. Potag.*, p. 14. 1883.

TABLE I.—YIELD OF INDIVIDUAL GLOBE ARTICHOKE PLANTS, 1916.

PLANT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
1916																																																		
June 30																													1										1							1				
July 1																																														1		1		
2								1															1																											
3																													1		1	2					1								1	1		2		1
4								1											1																															
5																													2		2	1			1		1		2			2		1	1	1		1		1
6			2					1				1	1			1			2				1	1																										
7																													2	1	1	1			1			1	2			1		2	1	1		2		2
8								3				1	1					1	1				1	2																										
9																												1	2	1	1	2			1	1	1	2	2		1	2	1	2	2	1		3		2
10			1									1											1	1																										
11																												1	1	2	2	1			1	1	2	1	2		1	1	1	2	2	2		2		1
12		1	2					1		1					1		1		1				3		1							1	1	1					1				1							
13																																																		
14						1	2					1							1				1		1			1	1				1		2	1	1	1	1			1	1					1		
15																																																		
16																																																		
17		1	1					1	2	1		1				1	1	1	3	1										1	1	1	1	1	2			1	2			3	5	1		1		1		1
18																																																		
19		1	1					1	1			1	1		1	2	1						1								1	3	2		3			2					2	2	2					
20																																																		
21																																																		
22		1	3				1					1	1		1				1		1		1						1		1				1	2	1	2	3	2		2	3	1	3	2		2	2	
23																																																		
24																																																		
25		2	1			1		2		2		1		1		1		1		1	1							1		2	1		1	1			2		1			1	1					2		
26																																																		
27		1						2	2					1			1															1	1		1	1			1	1		1	1		1	1		1		
Totals	0	7	11	0	0	2	3	13	5	4	0	8	4	2	3	5	4	3	10	2	2	0	10	4	2	0	0	4	11	7	11	13	7	3	13	6	9	10	18	3	2	14	16	11	13	12	0	18	2	8

However, there are only a very few sorts listed in our American seed catalogs, prominent among which are the Green Globe and the Purple Globe. The lack of distinctive names implies that the varieties have not been carefully standardized.

STATION STUDIES.

In February, 1913, two hundred fifty Green Globe artichoke plants were bought from a Southern firm. One hundred of the best plants were set in the garden in April. About twenty plants fruited that fall and these, together with twenty non-producers, were dug and stored in a cold house. In the spring of 1914 suckers from these old roots were potted and two rows placed in the garden. Ten of the fifty plants bore fruit. In 1915 the two rows yielded an abundant crop, some plants being peculiarly prolific, while others bore very few edible buds. These variations suggested taking careful records. During the first two or three years it was discovered that the artichoke not only needs winter protection but that it is particular in its requirements. A non-heating material — coal ashes — proved very satisfactory.

In the season of 1916 the plants of the two rows were given individual numbers and records kept of their performance. Table I shows at once the great variations that exist in the yielding capacity of different plants. The season was, however, peculiarly unfavorable to the artichoke, a crop which requires ample moisture during its fruiting season. The records do not, therefore, give a fair interpretation of the Globe artichoke's value. In normal seasons the yield is not only greater but also extends over a much greater period. Plate LXIII gives a view of the plants as grown at the Station.

CULTURE.

The Globe artichoke may be easily grown from seed either by starting the plants in a frame under glass or sowing the seed directly in the open ground. The seeds are large and germinate quickly, consequently young plants are easily grown. A pan of seedlings of the Green Globe variety is shown in Fig. 24. The objection to the seedage method of propagation is that it produces a great number of strains, similar in external appearance but differing in productivity. Propagation by means of suckers is the more satisfactory method of

Plate LXIII.— Two Rows of Globe Artichoke on Station Grounds, Showing Strong, Luxuriant Habit of Growth.

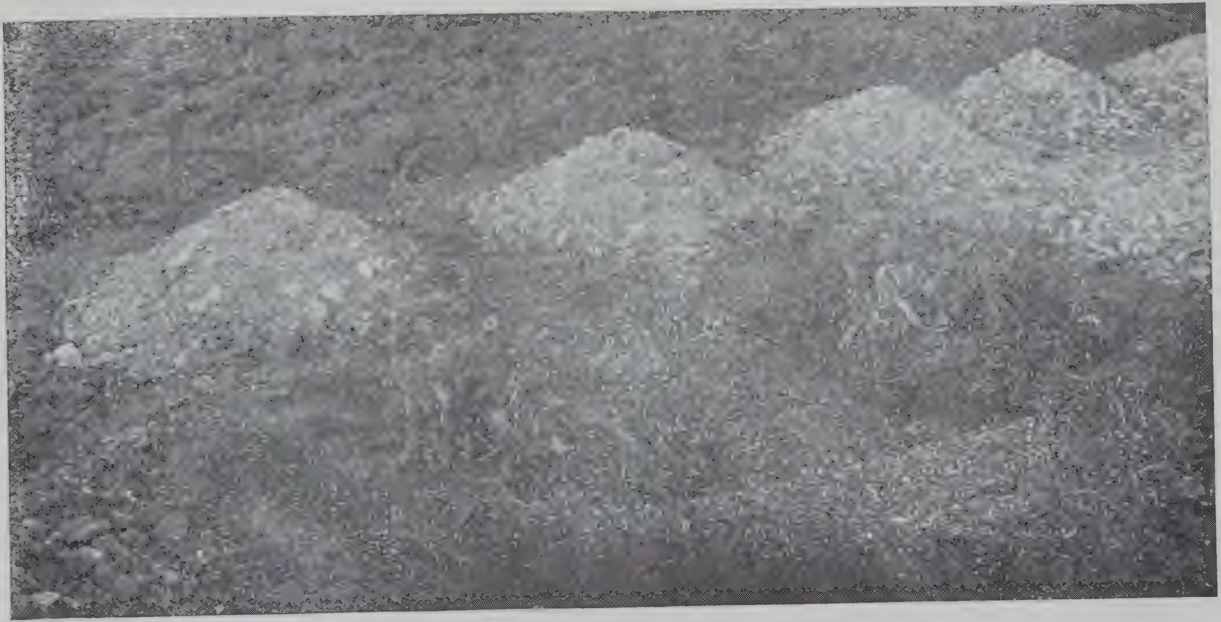

Plate LXIV.— Winter Protection of Globe Artichoke.
Two plants in foreground trimmed and ready for covering with coal ashes, as shown by mounds in background.

reproduction, for in this way fine yielding strains may be maintained and the varieties ameliorated. In a well-cared-for bed each plant may have sixteen to twenty of these suckers about the old root. In ordinary course of operation it is advisable to remove all but five or six of these young plants so that there is always abundant material for young stock. This method of propagation is best performed in the spring when the leaves have attained a length of twelve to fifteen inches. It is advisable to shovel away enough earth from the base of the plants so that the point of attachment to the old root may be seen. Then with a sharp spade or knife it is possible to cut away the young plant, together with a few roots (Fig. 25). These plantlets may be then set directly into a permanent row or may be nurtured under more favorable conditions and later transplanted.

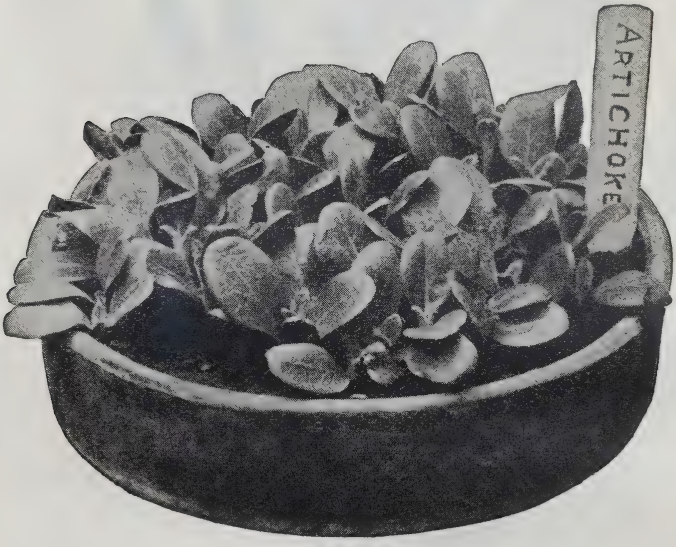

FIG. 24.— Artichoke seedlings at proper stage for first transplanting.

The distance between rows should be at least four feet and the distance between plants about three feet. In planning a bed it is well to provide for a three- or four-year duration; that is, renewing one row each year. The object of this arrangement is to dispose of the old plants as they become less fruitful and, more important still, to provide young plants which, with favorable environment, will send up fruiting stems in the later portion of the same season in which they are set, thus greatly prolonging the harvest period of the bed.

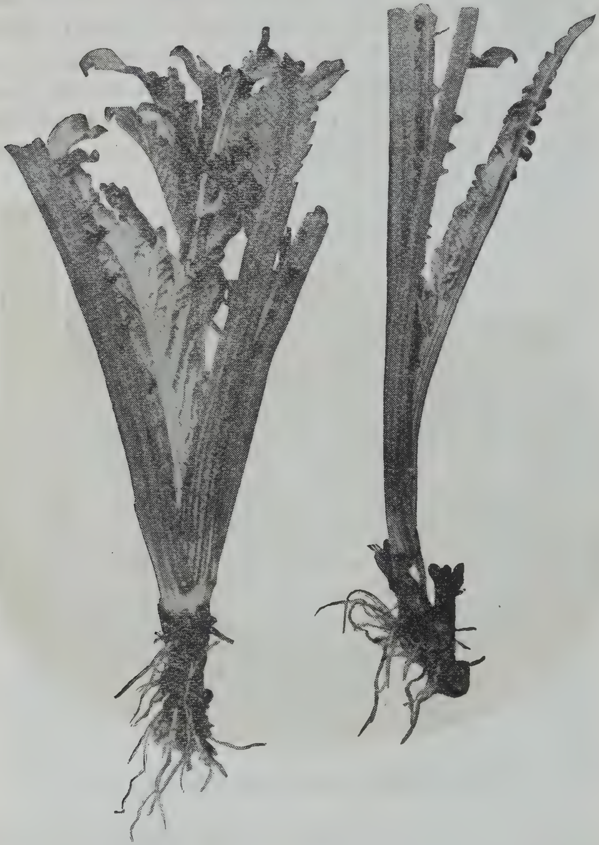

FIG. 2.—New plants from suckers. Strong leaves have been trimmed back to prevent drying. Small rootlets must be preserved in removing plants from parent.

The Globe artichoke is a gross feeder and requires an abundant supply of rotted manure in order to promote large crops. This manure should be dug into the soil between the rows and plants. Moisture is particularly essential during the fruiting season, but in normal seasons is plentifully supplied by rain and good tillage. Undoubtedly the artichoke would respond handsomely to irrigation.

The edible portion of the Globe artichoke is the globular flower bud which is produced on the terminal and side branches of a strong, upright fruiting stalk. This stalk stands above the foliage in height and, combined with the large ash gray leaves, gives the plant an attractive appearance. The heads are cut when they have attained their greatest size, prior to the actual appearance of the floral parts (Fig. 26). Exhibition heads may be secured by removing all except the terminal one and by supplying the plant abundant food and water. The fruiting stalks should be removed when all heads have been cut.

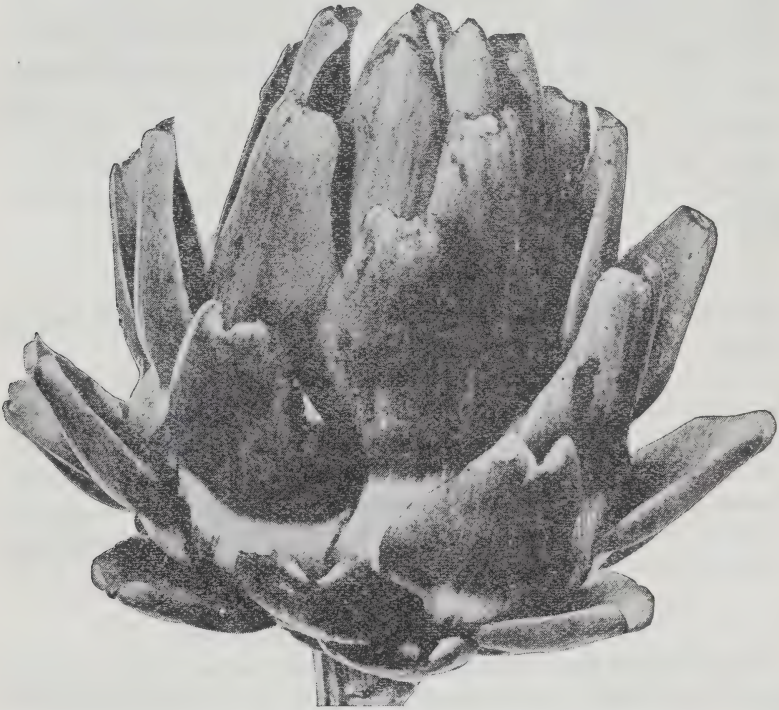

FIG. 26.— Edible portion of globe artichoke,— the flower bud. Bases of bracts and solid center, except spiny core, are eaten.

WINTER PROTECTION.

The Globe artichoke needs protection during the coldest part of the winter. Fresh manure was tried on the Station beds but with disastrous results in that, either by heating or by retaining too much water, it caused the heart of the crowns to rot, with consequent death of the plant. Coal ashes were then used, with success. The foliage is semi-hardy and should not be covered until late in November, and the covering removed in spring in late March or early April. Before mounding with ashes (Plate LXIV) the leaves must be cut back to within a foot of ground and drawn in about the crown. In the spring when the covering is removed the small leaves will be found to be in a blanched condition, but soon turn green and are followed by new leaves from the crown.

INSECT AND FUNGUS TROUBLES.

The plants on the Station grounds have been free from all fungus attacks.

A black aphid or louse is troublesome at times, literally covering the undersides of the leaves, and occurring especially during midsummer. This insect may be controlled by spraying thoroly with a dilution of Black Leaf 40 and whale-oil soap, the formula being 1 ounce of Black Leaf 40 and 4 ounces of whale-oil soap to $8\frac{1}{3}$ gallons of water. It is necessary to spray the lower surfaces of the leaves and about the crown of the plant.

USE OF THE GLOBE ARTICHOKE.

The artichoke must be considered a luxury rather than a staple. Both on account of this fact and on account of the large amount of ground space required per plant — 16 square feet — this vegetable has no place in a very small garden, especially in years of shortage of staple foods. However, to many people the artichoke is a much-desired delicacy and is eaten almost every day during fruiting season. The edible portion is the immature flower bud and which may be prepared for the table in many different ways. The following recipe is very satisfactory.

BOILED ARTICHOKES.

Whatever portion of the stem remains should be cut away from the bud, the large outside bottom leaves or bracts removed and imperfect ones trimmed. The heads should then be placed in cold water for one-half hour. Drain and cook one-half to one hour in salted boiling water to which has been added one tablespoonful of lemon juice or vinegar for each quart. When tender, drain and serve with melted butter, pepper, salt or any of the salad dressings. Of the large outer leaves only the small fleshy base is useful but the center leaves are tender almost to the tip. The spiny center called the "choke" is not edible. After the bracts and "choke" are removed the fleshy base remaining may be eaten.

INHERITANCE OF SEX IN STRAWBERRIES.*

R. D. ANTHONY

UNDER THE DIRECTION OF

U. P. HEDRICK.

SUMMARY.

The fact that several investigators are using the strawberry in a study of sex inheritance has made it seem advisable to publish certain data referring to this problem that have been secured during nearly thirty years of strawberry breeding at this Station.

The seedlings have been grouped under three classifications: perfect, imperfect and semi-perfect. A sharp line can be drawn between perfect and imperfect plants only after careful observation which, in doubtful cases, should extend through two seasons. The production of perfect blossoms is sometimes delayed till the plant is in nearly full bloom. Conditions leading to lack of vigor in the plant seem occasionally to suppress stamen development in perfect sorts throughout the entire season.

In general, there are two types of imperfect blossoms: those with stamens absent or rudimentary and those with filaments at least partially developed but anthers abortive if present.

Perfect varieties vary widely in the number of stamens and the amount of pollen they produce.

Pure male plants have been noted by others but none has been recorded among the thousands of plants grown at this Station.

Some Station seedlings have probably been classed incorrectly as imperfect but it is doubtful if such cases are numerous enough materially to influence the results.

When imperfect varieties have been pollinated by perfect sorts the resulting seedlings are of the two types in practically a 1 to 1 ratio.

The results secured when two perfect sorts have been crossed are: 2190 perfect, 9 semi-perfect, 5 imperfect. The five imperfects may be regarded either as representing errors in observation or as being the product of abnormal perfects.

The seedlings secured by self-pollinating perfect varieties differed materially from those from cross-pollinated perfects. There were 3159 perfect, 685 semi-perfect and 474 imperfect. All of the imperfects were secured from eight perfect varieties. The results with four of these are considered in a discussion of abnormal cases.

Of the seven cases in which abnormal results would seem to have been secured, five involved Marshall blood in varying degrees.

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* Reprint of Technical Bulletin No. 63, September, 1917.

Quality, when selfed, produced a larger proportion of imperfect seedlings than any other variety. One of these was tested in the greenhouse and produced no pollen even under such favorable conditions. When this seedling was pollinated by a perfect seedling of the same parent 31 perfect and 43 imperfect seedlings were obtained. When the seedling used as a male was self-pollinated all the progeny were perfect.

The results suggest that the best method of attacking the problem would be to grow pure seedlings of a considerable number of F_1 and F_2 seedlings of both Marshall and Quality.

As the further development of the problem of the inheritance of sex involves work not well suited to a department of horticulture it is being discontinued and the results published in their present incomplete form.

HISTORY.

Since the strawberry is being used by a number of investigators in the study of sex inheritance it has seemed advisable to publish certain data secured at this Station not so much because of the light they may throw on this subject but more to call attention to some of the problems involved.

Strawberry breeding was begun at Geneva in 1888. Of the many thousand seedlings produced since then nearly two thousand have seemed worthy of retaining beyond the first test but only twelve of these have finally been named and distributed. During most of this time the study of sex has been incidental but for the last four years an effort has been made to get more definite information than had previously been secured and especially to study certain crosses which seemed to give results at variance with those previously obtained.

TYPES OF PLANTS.

Through the early years all seedlings were grouped under two classifications: Perfect, those with both pistils and stamens, and imperfect, those with pistils only. During the last ten years those plants have been classed as semi-perfect the flowers of which possessed stamens but which produced so little pollen as to seem incapable of self-pollination.

One of the first results of this work was the belief that a sharp line can be drawn between perfect and imperfect plants only after careful observation of all blossoms throughout the season and in doubtful cases through a second season and that even then there is a likelihood of some plants being classified as imperfect which might produce pollen under more favorable circumstances. Not infrequently all of the opened blossoms on plants which are in nearly full bloom will have rudimentary or abortive stamens yet an inspection of buds just ready to open will show a full complement of functional stamens. Beyond doubt a number of plants were incorrectly entered in our early records as imperfect because of this delayed production of perfect flowers. In several cases in recent years plants which were recorded as imperfect in a field test have produced pollen and even formed perfect fruits under self-pollination when grown in the greenhouse with more favorable environment. Conditions leading to lack of vigor in the plant seem occasionally to suppress stamen development.

Imperfect blossoms can, in general, be divided into two groups: those in which the stamens are entirely absent or where the rudimentary filaments are represented by mere points, and those in which the filaments are present and at least partially developed but any anthers formed are abortive. It is in this second group that we will usually find those perfects which, because of abnormal behavior, have been incorrectly recorded as imperfect.

Among perfect varieties there is a wide range in the number of stamens produced and the abundance of pollen in the anthers. A final solution of some of the problems involved in the study of sex may make necessary the grouping of the perfects according to their pollen-producing power. This has not been done as yet at this Station.

Richardson [1] described pure male plants and seemed to find them produced rather commonly in certain of his crosses. No pure male has ever been noted among the thousands of plants grown at this Station.

GENERAL RESULTS.

In the following discussion it should be borne in mind that the term imperfect has probably been used in some cases where a more extended study of the individual would have proved it to be perfect. It is doubtful if such cases are numerous enough to influence the results to an appreciable extent. All the parents have been under observation long enough so that there is little doubt of the correctness of the terms used in describing them.

Imperfect × perfect: Summing up all crosses where imperfect blossoms have been pollinated from protected perfect blossoms, 1591 of the resulting seedlings were perfect and 1621 imperfect, practically a 1 to 1 ratio.[2] In the list of crosses entering into this total it will be noted that there are but few cases where the fluctuations from this ratio are extreme.

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[1] Richardson, C. W. A preliminary note on the genetics of *Fragaria*. *Journ. Genet.* 3:171–177.

[2] Attention should be called to certain unpublished results of two other investigators. Of the 1013 seedlings obtained by W. C. Stone at the Vermont Station by crossing imperfects with perfects, 659 were imperfect, a ratio of 1 to 1.86. From the Ontario (Canada) Agricultural College Prof. J. W. Crow reports the following:

Warfield (imp.) x Parsons	211 perfect, 122 imperfect
Buster (imp.) x Williams	144 perfect, 140 imperfect

It is possible that all varieties do not behave alike in this type of cross and that the varying results are due to factors other than experimental error. The behavior of Marshall and Quality as shown later in the report lends color to this idea.

TABLE I.— SEX OF PLANTS FROM CROSSING IMPERFECT BY PERFECT VARIETIES OF STRAWBERRIES.

	Perfect.	*Imperfect.*
Autumn x Brandywine	58	51
Autumn x Chesapeake	12	18
Autumn x Marshall	81	80
Autumn x Prolific	83	71
Bubach x Laxton Noble	23	23
Bubach x Van Deman	16	6
Columbia x Atkins Continuity	78	71
Columbia x Pan American	47	47
Columbia x St. Joseph	53	58
Crescent x Bomba	10	12
Crescent x Dawley	13	9
Crescent x Gov. de Bossult	19	4
Crescent x Feicht No. 2	46	58
Crescent x Lennig White	67	90
Crescent x Jessie	2	2
Crescent x Old Ironclad	28	42
Crescent x Sharpless	45	48
Daisy x Lennig White	4	3
Eureka x *Fragaria virginiana*	2	3
Eureka x Van Deman	6	3
Feicht No. 3 x Feicht No. 2	28	47
Haverland x Jessie	4	3
Haverland x Van Deman	4	4
Hunn x Atlantic	31	48
Hunn x Marshall	97	63
Johnson Late x Dawley	1	2
Johnson Late x Lennig White	27	28
Johnson Late x Sharpless	8	11
Lida x Lennig White	6	3
Lida x Dawley	13	9
Middlefield x *Fragaria virginiana*	24	33
Middlefield x Lovett Early	16	17
Middlefield x Station No. 208	13	12
Middlefield x Van Deman	27	18
Mrs. Cleveland x Lovett Early	12	18
Mrs. Cleveland x Prince of Berries	1	2
President x Marshall	399	413
Sample x Marshall	6	11
Sta. No. 26 (Crescent x Old Ironclad) x Sta. No. 147 (Lida x Lennig White)	44	46
Sta. No. 77 (Crescent x Lennig White) x Sta. No 62 (Crescent x Lennig White)	4	2
Sta. No. 95 (Crescent x Dawley) x Sta. No 208 (Johnson Late x Sharpless)	53	54
Sta. No. 198 (Johnson Late x Sharpless) x Sta. No. 208 (Johnson Late x Sharpless)	3	2
Sta. 1402 (Quality pure) x Sta. 1403 (Quality pure)	*38	43
Stayman No. 1 x Bomba	15	15
Stayman No. 1 x *Fragaria virginiana*	16	7
Stayman No. 1 x Jessie	1	3
Stayman No. 1 x Sharpless	4	1
Summit x Bomba	3	7
	1591	1621

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* With seven of these the sex was marked as uncertain.

Perfect × *perfect:* The following is a list of the cases where perfect blossoms have been emasculated and pollinated by other perfect sorts:

TABLE II.— SEX OF PLANTS FROM CROSSING PERFECT BY PERFECT VARIETIES OF STRAWBERRIES.

	Perfect.	*Semi-perf.*	*Imp.*
Brandywine x Pan American	3	....	....
Brandywine x Sta. 1570 (President x Marshall)	270	....	....
Chesapeake x Atkins Continuity	173	....	....
Chesapeake x Marshall	45	....	....
Chesapeake x Pan American	170	....	....
Chesapeake x St. Joseph	79	....	....
Gov. Fort x Sta. 1551 (President x Marshall)	177	2	....
Honey x Bederwood	6	....	....
Honey x *F. Chiloensis*	12	....	....
Marshall x Atkins Continuity	140	....	....
Marshall x Pan American	86	....	....
Marshall x Prolific (Sample x Marshall)	118	2	....
Marshall x St. Joseph	95	1	....
Marshall x Sta. 1458 (Prolific pure)	134	....	....
Michel Early x Lennig White	8	....	....
Progressive x Superb	19	....	....
Prolific x Atkins Continuity	9	....	....
Prolific x Marshall	95	....	....
Prolific x Gov. Fort	155	3	3
Prolific x St. Joseph	101	1	1
Sta. 1458 (Prolific pure) x Marshall	182	....	....
Sharpless x Wilson	14	....	1
Superb x Progressive	99	....	....
	2190	9	5

Since the nine semi-perfect plants produced some pollen, in this discussion they should be grouped with the perfects. The five recorded as imperfect may be looked upon in two ways; either as representing errors in observation or as being the product of abnormal perfects. This letter view will be considered later.

Throwing aside the few abnormal cases we would seem justified in assuming from these results that seedlings resulting from a cross of two perfect varieties will be perfect.

Perfects selfed: A number of the perfects which entered into the preceding table, as well as a few other varieties, have been selfed. From these one would expect results comparable with those secured when perfect varieties were inter-crossed. The results were quite different. There were 3159 perfects, 685 semi-perfects[1] and 474 imperfects. The list of perfects which have been selfed is given in Table III.

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[1] The high proportion of semi-perfects as compared with those in the two preceding types of crosses is probably largely due to the fact that most of the selfing of perfects has been done since the plan was adopted of grading certain seedlings as semi-perfect. This is not sufficient, however, to account for all of the increase.

Table III.— Sex of Plants from Self-pollination of Perfect Strawberry Varieties.

	Perfect.	*Semi-perf.*	*Imp.*
Amanda	277	5	....
Bederwood	3	....	....
Brandywine	18	....	....
Burt	4	....	....
Charles Downing	34	....	10
Captain Jack	2	....	1
Golden Defiance	3	....	....
James Vick	25	....	2
Joe	272	24	....
Marshall	700	36	10
May King	3	....	....
Mrs. Garfield	2	....	....
Prince of Berries	14	....	7
Progressive	49	....	....
Prolific [Sample (imp.) x Marshall (perf.)]	920	564	391
Quality [Hunn (imp.) x Atlantic (perf.)]	48	24	43
Senator Dunlap	507	14	....
Sta. No. 1402 (Quality pure)	45	....	....
Sta. No. 1458 (Prolific pure)	69	....	....
Sta. No. 1466 (Prolific pure)	96	14 4?	5 5?
Sta. No. 1551 (President x Marshall)	55	....	....
Superb	13	....	....
	3159	685	474

As stated before, we may group the semi-perfect plants with the perfects since they possessed functional male organs. This leaves eight perfects recorded as producing imperfect seedlings. The work involving Charles Downing, Captain Jack, James Vick and Prince of Berries was done more than twenty-five years ago when but meager attention was paid to the record of sex and as there are but little data available and the total number of seedlings involved is relatively small, it would seem best to drop these from the discussion. The remainder are considered in the following paragraphs.

ABNORMAL CASES.

In the following cases results have been secured which would seem to be abnormal when compared with other seedlings from similar crosses:

	Perf. + Semi-perf.	*Imp.*
Perfect x Perfect.		
Prolific x St. Joseph	102	1
Prolific x Gov. Fort	158	3
Sharpless x Wilson	14	1
Perfects selfed.		
Marshall	700	10
Prolific	1484	391
Quality	72	43
Sta. 1466 (Prolific pure)	114	5 5?

With the exception of the one imperfect from the cross of Sharpless by Wilson and those from Quality all these involve Marshall blood in varying degrees since Prolific is a Station seedling obtained by crossing Sample with Marshall.

The breeding performance of Marshall and its seedlings on the Station grounds is given in outline below:

	Per. x Semi-per.	*Imp.*
Marshall pure	736	10
Autumn (imp.) Hunn (imp.) President (imp.) Sample (imp.) } x Marshall	585	558
Chesapeake x Marshall	83	......
Marshall x { Atkins Continuity Pan American St. Joseph }	322	......
Prolific x Marshall	95	......
Marshall x Prolific	120	......
Marshall x Sta. 1458 (Prolific selfed)	134	......
Sta. 1458 x Marshall	182	......
Prolific selfed	1484	391
Sta. 1458 (Prolific selfed) selfed	69	......
Sta. 1466 (Prolific selfed) selfed	114	5 5?
Prolific x St. Joseph	102	1
Prolific x Gov. Fort	158	3

A larger proportion of the seedlings of Quality is imperfect than with any other variety. Quality was a Station seedling obtained by crossing Hunn, an imperfect Station seedling, with Atlantic. The variety was discarded before attention was called to its abnormal production of imperfect seedlings but two of its pure seedlings were still in the Station plantation, one perfect and one imperfect. Even under the most favorable conditions in the greenhouse the imperfect seedling did not develop anything more than rudimentary stamens. The perfect seedling was selfed and the imperfect pollinated by the perfect. The results follow:

Station 1403 selfed	45 perf.	0 imp.
Station 1402 x Station 1403	31 perf.	43 imp.

Although the results from the imperfect seedling pollinated by the perfect are somewhat wide of the 1:1 ratio which seems to be the rule with such crosses, nevertheless the difference is no greater than with a number of such crosses as will be seen by referring to Table I.

The above results fail to furnish any ready answer as to the cause of the abnormal production of imperfect seedlings. The behavior of Prolific and its pure seedlings suggests that the best method of attacking the problem would be to grow selfed seedlings of a considerable number of F_1 and F_2 seedlings of both Marshall and

Quality.[1] To do this would involve much time and a large area; and the value of the results would not seem to justify this department in continuing the work, since its chief problems are more strictly horticultural in nature. For this reason it has seemed best to publish the data in the present incomplete form so that they may be available to other workers.

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[1] Attention should again be called to some results secured by Prof. Crow. Of more than 1700 seedlings which he obtained by crossing Parsons with eleven other perfect sorts, 34 were imperfect. These 34 were all from a single cross, Parsons x Howard 17, a cross which gave 381 perfects. Also, in a group of seedlings from William Belt x Parsons one imperfect was found.

ORCHARDS: LOCATION AND CARE.*

U. P. HEDRICK.

The management of an orchard is not a matter to be settled by one man for another. To tell a man exactly how to grow fruit is impossible. But there are fundamentals in fruit-growing as in all professions and these can be set forth. To call attention briefly to some of the fundamentals of orchard management is the office of this circular.

LOCATION.

As to markets.— Choice depends upon whether the grower intends to sell in the wholesale or the retail market. If wholesale, he may be across the continent or the ocean from his market and may make his choice depend only upon the means of shipping and the cost. The more means of transportation, the greater the competition in rates and the more markets to be reached. For the retail market, the nearer and the more ways of reaching it, the better.

As to soil.— All fruits may be grown on a wide range of soils by exercising judgment in selecting varieties and in handling the soil. Fruit of some kind can be grown on any soil suitable for farm crops. Land upon which wheat, oats and potatoes thrive is usually good fruit land. The character of the soil greatly influences the quality and the quantity of the product. Select soils suitable for the fruit and the varieties to be grown.

As to site.— The ideal orchard-site is one somewhat above the surrounding country so that there be atmospheric and soil drainage. Cold air settles to low areas making low sites frosty, damp and cold in winter. High lands need not be rolling, it being sufficient if there be adjacent lower lands. It is not safe to plant in a pocket. Locations subject to fogs are not suitable for growing fruit.

As to exposure.— The exposure of an orchard is determined by the direction of the slope of the land. Exposure exerts great influence on the warmth of the soil and upon the force of the wind, and is of the utmost importance in the location of orchards of tender fruits. General suggestions are as follows: 1st. Near a body of water, choose the exposure toward the water. 2nd. Between the two sides of a river or lake, the side exposed to the prevailing wind is freest from frost. 3rd. Away from water a northern exposure suits tender fruits, since on such sites the blooming period is retarded. 4th. A southern or southeastern exposure hastens maturity.

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* Reprint of Circular No. 52, May 10, 1917.

As to cold.— Cold injures plants in two ways — by frosts and freezes and by extremes of winter temperature. Frosts occur on still, clear nights and are more or less local. Freezes are usually preceded by storms and are general in range. Frosts may be avoided by the selection of a location but freezes are beyond the control of man except when comparatively light, when they can sometimes be counteracted by means of orchard-heaters. Immunity to cold is best secured by planting near a body of water.

As to water.— Bodies of water equalize temperature since water does not respond to fluctuations of temperature as quickly as land. The effects of water on climate are: In the spring a body of water lowers the temperature and thus helps to hold back vegetation until danger from frost is past; in the summer it cools the days and warms the nights; in the fall it lengthens the growing season and keeps off autumn frosts. The larger and deeper the body of water the greater the influence, depth being of more importance than exposure of surface.

As to air-currents.— Winds are beneficial when they bring warmer air and when they keep frosty air in motion. Strong winds injure plants by blowing them over, breaking tops and by blowing off fruits. Cold winds check growth and give conditions for some diseases. In dry regions winds take moisture from soil and plant to the detriment of the latter. Openness to winds, or protection from them, must be secured by choice of location.

ORCHARD PLANS.

Trees may be set in squares, equilateral triangles or quincunxes.

Squares.— Setting in squares is the simplest and the commonest method. The objection urged against the square is that the trees are not equidistant from each other. But plowing, cultivating, spraying and harvesting are carried on most conveniently in orchards laid out in squares; and in time, roots and branches utilize all the space in a square. The maximum number of trees an orchard can efficiently support, can be planted in squares.

Equilateral triangles.— In this method the trees are placed in triangles and are equidistant from each other thus dividing the ground equally between the trees. This plan admits of 15 per ct. more trees to the acre than does the square and permits cultivation in three directions.

Quincunxes.— In the quincunx plan the orchard is planted in squares with a tree in the middle of each square. The quincunx is used when it is desired to plant fillers to be removed when the trees begin to crowd. This plan gives nearly double the number of trees to the acre.

Laying out an orchard.— In planting in squares, survey the orchard on two adjacent sides leaving a conspicuous stake at the location of each tree. It is then easy to place each tree accurately in the remainder of the orchard with a line and by sighting. In planting in triangles, use an equilateral triangle of wood or wire, of dimensions equal to the distance desired between trees, being guided for the first rows by a line of stakes.

Fillers.— The practice of planting temporary trees between rows of permanent ones in an orchard has much to recommend it. These filler trees can be used to advantage only when the permanents are set in squares. Then a filler may be put in the center of each square, giving nearly double the number of trees per acre; or fillers may be set both ways between the permanents giving three times as many fillers as permanents. Quick-bearing varieties, as Wealthy, Oldenburg, Twenty Ounce and Rome, are all good filler sorts. Some plant cherries, peaches or pears as fillers but any of these in an apple orchard so complicate orchard operations, especially spraying, that they usually prove unsatisfactory. The fillers must be cut out as soon as they crowd the permanent trees.

PLANTING.

Preparing the soil.— All subsequent treatment fails if the preparation of the soil is imperfect. The requisites are: 1st. Good drainage should be provided. 2nd. Hard, sour, infertile spots should be brought into uniform condition with the rest of the orchard. 3rd. The ground should be deeply plowed and then thoroly fitted with pulverizing tools.

Choosing varieties.— 1st. Have a definite idea of the purpose for which the fruit is to be grown — whether for wholesale or retail, canning, evaporating or home use. 2nd. Varieties have adaptations to localities — plant the fruit and the variety which grow best in the locality. 3rd. Choose with reference to inter-pollination — some fruits are self-sterile and must have varieties which will fertilize them. 4th. For commercial plantings, choose standard varieties.

Distance between trees.— The distance apart at which trees should be set depends on the variety, the soil and the method of pruning. Trees are oftener set too close then too far apart. The notion that trees should be set only far enough apart so that branches will not overlap is wrong, as the roots of a mature tree spread farther than the branches, and should not compete for food and moisture. Cultivation, harvesting and spraying can be well done only when trees are at a proper distance apart. Average distances of planting are:

Apples...........	35–45 feet each way	Pears.........	18–20 feet each way
Cherries..........	16–22 "	Quinces.......	12–16 "
Peaches..........	18–22 "	Plums.........	18–20 "

Selecting trees.— Select trees of average size for the age, choose plants typical of the variety. Trees grown near home are usually better than those brought from a great distance. Every precaution should be taken in buying to insure trees true to name and free from pests. Other things being equal, a short, stocky tree is better than a tall, spindling one; one with many branches is better than one with few; and always the root-system should be well developed.

Pedigreed trees.— Some believe that the variations in fruit and trees to be found in varieties can be reproduced by taking cions or buds from the plants possessing the variations. A few nurserymen offer trees for sale with a " pedigree " to show that they come from good ancestry. There is, however, little evidence to show that any sort of fruit has come into existence by selection; that any variety has been improved, or that any variety has degenerated through natural or artificial selection. Fruit-growers, therefore, should steer clear of " pedigreed stock " and " improved strains " of varieties until the new product can be seen by competent judges growing side by side with the parents.

Number of trees per acre.— When trees are planted in squares the number per acre is determined by multiplying the distances between the trees each way and dividing 43,560 (the number of square feet in an acre) by the product. When planted in quincunxes, double the number for squares. When planted in triangles, add fifteen per ct. to the number required to set the area in squares.

Age to plant.— The age at which a tree should be planted is governed by the kind of fruit, the variety, the climate and the soil. In New York, generally speaking, all trees should be two years from bud excepting the peach which is best set as a one-year-old.

Time to plant.— In mild climates the most favorable time to transplant is in late fall at the beginning of the dormant period, as the roots will then have time to heal their wounds, and to form new rootlets for the next growing season. But in the cold climate of New York trees should be set in the spring as there is then far less danger of winter injury, not because of less tender roots but because the soil, having been disturbed, is more porous and the frost strikes harder.

Dynamite in digging holes.— Dynamite is being widely advertised for use in digging holes for trees. There is little evidence to show that trees in the average soil thrive better in holes made by using dynamite and until such evidence is forthcoming it is better that the holes be dug. It is quite as probable that harm rather than good will be done through the use of explosives since the explosives may mix poor sub-soil with good top-soil. Sometimes, dynamite may be used to advantage in breaking the hardpan if it is too near the surface. Dynamiting costs more than digging.

Top-working young trees.— Setting a thrifty variety of apple or pear and then grafting or budding on the top a weaker variety, has some advocates. This top-working is probably a procedure worth while only with a very few varieties. In general, the chances of getting malformed, lopsided trees and of delaying the bearing period are so great that top-working can be recommended only for a very few sorts that seem difficult to grow on their own trunks. Topworking is best done in the nursery.

Pruning at transplanting.— In transplanting it is necessary to cut away part of the branches to enable the injured root system to supply the top with sufficient food and water. The less the roots are injured, the less the top need be cut away. The top buds on a branch develop soonest and produce the largest leaves. Now a newly set tree will grow best if it can develop a large leaf surface before dry, hot weather sets in, and this it will do if some branches are left intact with the top buds. Therefore, instead of shortening all branches, cut away some of the branches entirely. The tree so pruned will start growth and acquire vigor more quickly. If there be no surplus branches, shorten in all branches. The peach is an exception and young trees of this fruit start best when surplus branches are removed and the remainder are cut back to spurs of three or four buds.

Care of shipped trees.— If not ready to plant when the shipment arrives, the trees should be heeled in. To heel in, dig a trench a foot or more deep and wide enough to receive the roots. In digging, use the soil to make a sloping bank on the south side of the trench. Spread the trees in a sloping position with the roots well down in the trench. Cover the roots and about half of the bodies of the trees with the soil, working it in firmly about the roots.

Setting.— The roots should be exposed as little as possible to sun and wind. In planting, keep the trees in the field covered with earth or a wet sack until they are put in the ground. The chief care in setting should be to place a moist, firm soil in close contact with all of the roots of the plant. The trees should be set a little deeper than they stood in the nursery row. Mulching after planting is excellent as it prevents evaporation and maintains an equable temperature most favorable to root growth. Mulching is best done by frequent, shallow cultivation. Water thoroly or not at all. Newly set trees should not be heavily fertilized as rich foods placed in the soil cannot be utilized by the root system until all injuries have been healed.

CULTIVATION, COVER-CROPS AND INTER-CROPS.

When to cultivate.— Cultivation should be started early in the spring to save the winter's accumulation of moisture and to warm up the soil so that the plants can quickly start growth. Cultivate at intervals of two weeks until mid-summer and then stop, as the trees have by this time completed growth and the wood must mature for winter. Sod-mulch orchards are not recommended for New York except on hilly lands.

How to cultivate.— The land should be turned deeply with a plow early in the spring, or on heavy soils in late fall, and in such a way as to secure level culture. Afterwards the cultivation should be shallow but deep enough to break the crust and kill the weeds. The implements should be adapted to the soil and to the work, and may be any of the various clod-crushers, harrows, cutaways or weeders that do the work well in the particular piece of soil. Tools with high handles or high wheels are to be avoided. Harnesses without projections, and short single-trees should always be used.

Crops in the orchard.— Hoed crops and no others should be grown in an orchard and these for the first few years only — hay or grain, never. Whatever the crop, an open space of several feet should be left about each tree. In general, the crop should be low-growing, as beans, cabbage, potatoes and other truck crops. Nursery stock should not be grown in an orchard. All cropping must cease when the orchard begins to bear. Truck crops should be given preference over small-fruits.

Cover-crops.— A crop sown in mid-season may be made valuabl in the following ways: To improve the physical condition of th land; prevent hard soils from packing; hold rain until it has had tim to soak into the land; dry out the soil in the spring; catch and hol the nitrates; add humus to the soil; to enrich the soil; and to haste the maturity of the wood. The legumes, among which are clover, peas and vetch, make the best cover-crops. Other crops are oats, rye, buckwheat, turnips and corn. The choice depends on the soil and the kind of fruit. A rotation may be practiced or a mixtur of the above crops used. The cover-crop should be sown in midsummer at the time of the last cultivation and be plowed under lat in the fall or very early the next spring. The grains — oats, rye o barley — should be sown at the rate of two bushels per acre; clovers, fifteen pounds per acre; vetch, twenty to forty pounds per acre.

FERTILIZERS FOR FRUITS.

If the land is well drained, well tilled and well supplied with organic matter, commercial fertilizers are needed little in most New York orchards. The exceptions are found in sandy and gravelly soils and in soils too wet or too dry. No doubt there are many orchards that might be benefited by an application of one of the chief elements of fertility; some may require a complete fertilizer.

How may a fruit-grower know whether his trees need fertilizers? — It may be assumed that, if trees are vigorous, bearing well and make a fair amount of new wood each season they need no additional plant food. If the trees are not in the healthful condition described, the logical thing to do is to look to the drainage, tillage and health of the trees first and to the more expensive and less certain fertilization afterward. Even as a last resort, fertilizers ought not to be used for trees unless the owner has positive evidence that his soil is lacking in some of the elements of plant food. To obtain such evidence a fruit-grower should carry on a fertilizer experiment.

A fertilizer experiment.— Select a portion of the orchard as uniform as possible in soil and varieties. Use at least five trees for each plat and on different plats fertilize as follows: (1) Acid phosphate, 13 pounds of 14 per ct. to each tree if they stand 40 feet apart; (2) muriate of potash, 8 pounds per tree; (3) phosphate and potash as above; (4) phosphate and muriate as above and 13 pounds of medium grade dried blood and $3\frac{2}{3}$ pounds of nitrate of soda per tree; (5) six tons of good stable manure to the acre, or 400 pounds per tree, should be applied on a fifth plat, and (6) a similar plat should be left unfertilized for a check.

What fertilizer should be used in an orchard?—The fertilizers named in the preceding paragraph, in the quantities given, for each of the chief elements, phosphorus, nitrogen, potassium are probably as good as any. There are, however, no objections to the use of other fertilizers containing equivalents of the three chief elements. There is little or nothing to show that lime is of benefit in an orchard though it may be of much benefit to cover-crops grown in the orchard.

Application of fertilizers.—Fertilizers should be applied in the spring as soon as the ground can be worked, spreading them about the trees over an area somewhat greater than that covered by the spread of the branches. Apply manure before plowing; fertilizers immediately after.

PRUNING.

Pruning young trees.— In pruning a one-year-old tree, remove the branches and cut back the remaining whip to the height desired. If the tree is two years old, as is usually the case with all fruits excepting the peach, or when the one-year-old plants have been set a year, the real work of shaping the tree may be begun. This early pruning is more or less provisional though an ideal for the future tree must be plainly in the mind at the time.

Height of head.— Shall the tree be low or high headed? Usually the choice should be for a low-headed tree: for a low-headed tree is more easily sprayed and pruned; the fruit is more readily thinned and harvested; crop and tree are less subject to injury by wind; the trunk is less liable to injury by sunscald or winter-killing; the top is more quickly formed; and the low-headed tree bears fruit quicker. No advantage as to cultivation is gained by either method over the other, as a well-trained tree with a low head permits the cultivator to come sufficiently near the tree. By " low headed " is meant a distance from earth to the first limb of from one to three feet. The peach may be headed at the lower distance, the plum, pear and cherry somewhat higher, while the apple should approach the upper limit.

Two types of top are open to choice.— These are the vase form or open-centered tree, and the globe or close-centered tree. In the first the frame-work of the tree consists of a short trunk surmounted by four or five main branches ascending obliquely. In the close-centered tree the trunk is continued above the first whorl of branches, forming the center of the tree. There are several modifications of

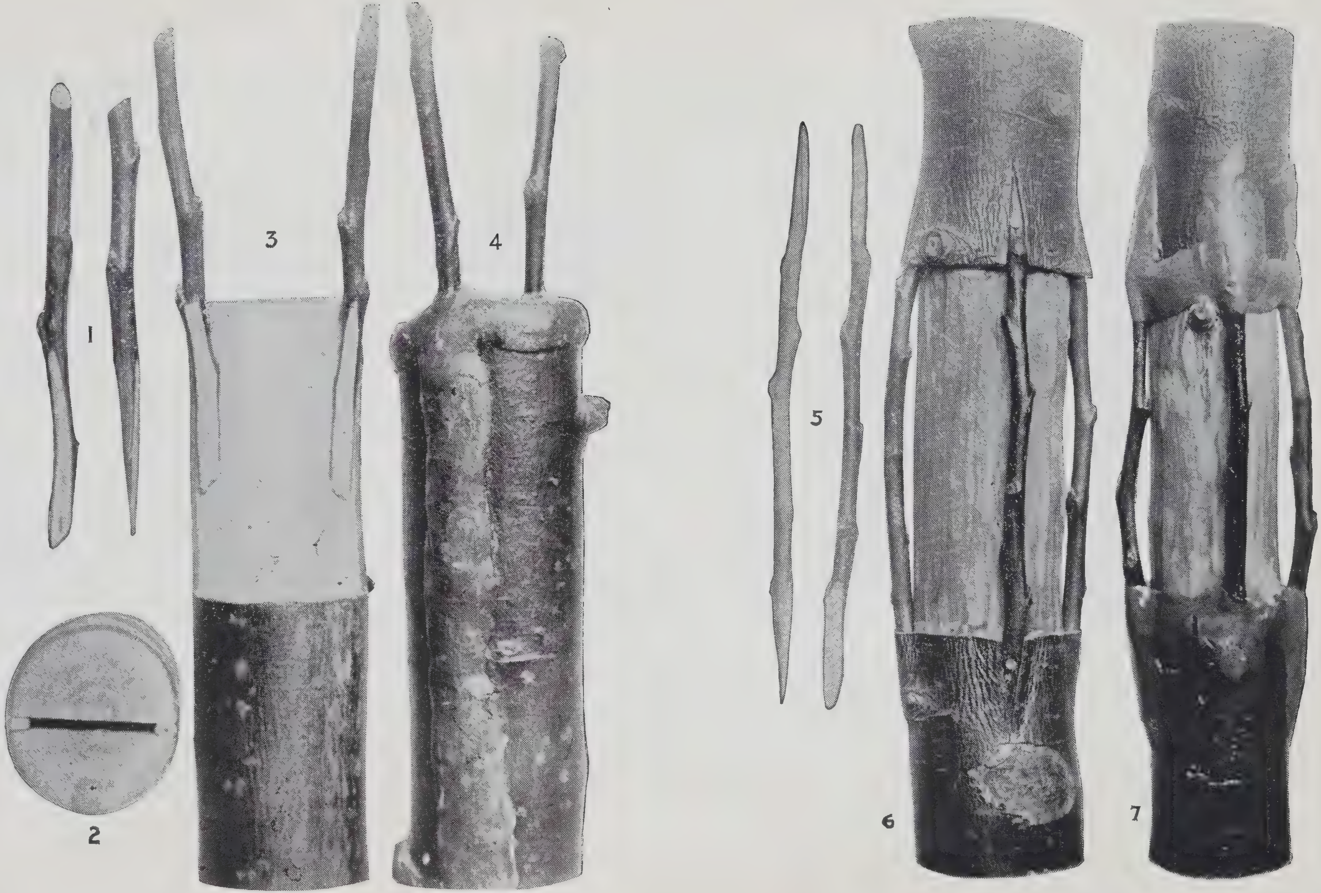
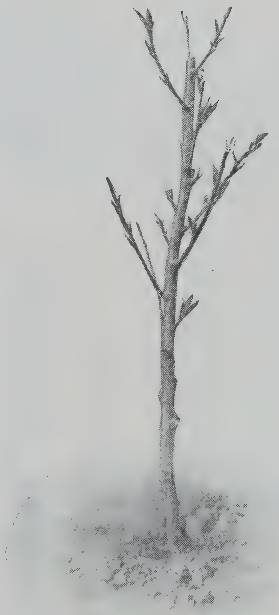


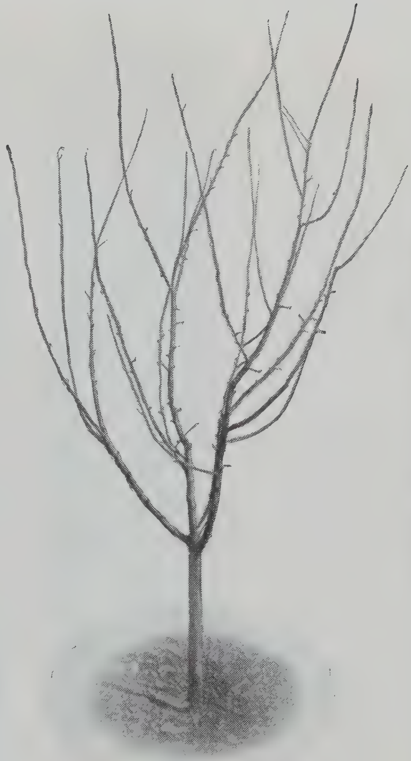
Plate LXV.— Cleft and Bridge Grafting.

1, Cions prepared for cleft grafting; 2, cross-section with cions in place; 3, cions properly placed; 4, completed, waxed cleft graft; 5, cions prepared for bridge grafting; 6, cions properly placed and secured; 7, completed, waxed bridge graft.

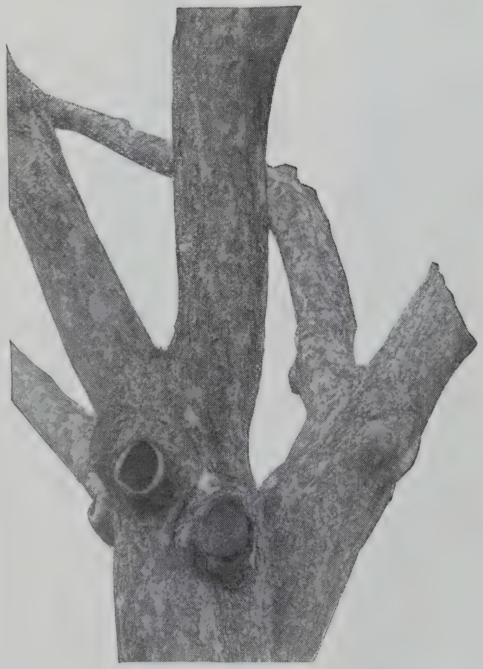
Plate LXVI.



A peach tree pruned at planting.



A young Baldwin with a well-formed head — planted two years.



Properly made wounds in pruning.

each of these. In New York the open-headed, vase-formed tree is best for the peach and the close-centered two-story tree is best for all other fruits. Whatever the form, care should be taken that the lowest branches are longest, so that the greatest possible leaf-surface will be exposed to the sun and light that close crotches and closely over-lapping branches be avoided.

Young trees need to be pruned only to train the tree.— Just how much to prune young trees depends upon the fruit, the variety, the soil and the climate. Fruit-growers usually prune trees too much. If trees are originally well selected, all that is needed is to remove an occasional branch which starts out in the wrong place — the sooner done the better — and to take out dead, injured or crossing limbs. Weak, sickly trees, or ones making long, willowy growth may require somewhat more severe pruning.

Pruning to increase vigor.— When a tree is bearing many small fruits, when the top contains dead branches, or when the seasonal growth is short and scant, it may be taken for granted that the tree lacks vigor. Such trees may usually be reinvigorated by judicious pruning. In professional terms the tree must be " pruned for wood." This consists in cutting back a considerable number of branches and in wholly removing others. Such pruning should usually be extended over two or more years. In pruning to increase vigor, the following rules are usually applicable:

1st. Weak-growing varieties may always be pruned heavily; strong-growing kinds lightly. 2nd. Varieties which branch freely need little pruning; those having unbranching limbs should be pruned closely. 3d. Rich, deep soils favor growth; prune trees in such soils lightly. In shallow, poor soils, trees produce short shoots; cut the wood closely.

In pruning, consider the habit of the tree.—When trees have a spreading, drooping or long, slender habit of growth, prune to buds that point upward or to the center of the plant. If the habit be upright and dense, cut to lower or outer buds and so spread the compact top. The " off year " habit of bearing is intensified by spasmodic and severe pruning. Prune biennial bearers lightly and yearly. The experiment may be tried of pruning only before the fruiting season as a means of correcting over-bearing and alternate bearing, faults which usually go together. The heads of all young trees may be left fairly dense, for when the trees come in bearing the weight of the crop opens the head; meanwhile by saving the foliage you have obtained a larger trunk and more bearing wood.

Heading-in, or cutting back all of the branches on a tree, makes the the top of a tree thicker and broader.— In New York heading-in is practiced regularly only with peaches and some plums. Since heading-in is usually practiced to reduce extensive growth it is always best to consider if there is not some way of preventing too much growth; as, thru withholding fertilizers, restricting winter pruning, or by use of cover-crops. In other words, it is better not to force trees than to force them to produce too much wood and then have to prune heavily. Peaches and some plums bear fruit on the wood of the past season so that the crop is borne progressively farther away from the trunk. It is necessary to head-in these fruits to keep the bearing wood near the trunk. Apples, pears, most plums, and cherries are borne on spurs from wood two or more years old, and therefore, with these, heading-in is not needed as a regular practice.

Suckers.— When trees are severely pruned, a growth of long, vertical shoots with few leaves often follows. These are suckers or water-sprouts and should be removed whenever and wherever found excepting that occasionally they may be used in the development of normal branches. Suckers may also come from over-manuring or other causes which upset the normal growth of the tree.

Dehorning condemned. — By dehorning is meant the cutting back of all branches to a short distance from the trunk. There are exceptional cases when this wholesale removal of the top is rational, as when good trees in their prime are severely injured by overbearing, heavy winds, snows, fungi or other causes. But to "dehorn" old trees for the sake of one or a few more crops is usually poor practice. Such trees in most cases should go to the brush heap and a young orchard be planted to take the place of the old. The tops of very high trees may be lowered several feet but this is not dehorning.

Hints on pruning.— There is much opportunity for choice of ladders, knives, saws and shears. Wounds over three or four inches in diameter seldom heal and decay sets in, caused by wood-destroying fungi, and these, with the action of the weather, are followed by rotten wood, a hollow branch and a diseased tree. The cut should be made close to the trunk so as not to leave a projecting stub. A coating of lead paint protects the cut surface from moisture but it is a waste of time to paint wounds less than three inches in diameter. Experiments at this Station prove that, on the apple, wounds heal more quickly if not painted.

Time to prune.— Pruning is often left to "time and chance" but there is a best time, which is winter when the wood is dormant. Trees may be pruned from the time the leaves drop in the fall until they start in the spring.

PESTS.

Insects.— Insects injure plants in two ways: By eating parts of the plant, as do caterpillars and beetles; and by sucking the juices from plants as do scale insects and plant lice. Depredators of the first class are killed by the application of poisons which the insect must take internally. For the second class preparations are used which kill by contact with the insects as tobacco, lye, some oils, soap, pyrethrum, sulphur and lime-sulphur.

Fungi.— These are characterized, as they occur on fruit-plants, by definite spots, discolorations or excrescences scattered over the leaf, fruit or branch; as, apple-scab, black-knot, leaf-blight. After a fungus gets into the tissues of the plant it is difficult to kill it so that treatment should be preventive rather than remedial. To prevent fungi from gaining a foot-hold on plants, the parts exposed to attack must be coated with a thin film of some substance which is destructive to the fungi. The best of these substances for most fruit diseases are lime-sulphur and bordeaux mixture.

Bacteria.— Some diseases are caused by bacteria; as pear-blight, and possibly peach-yellows and little-peach. There are no specific treatments for bacterial diseases. Orchard sanitation in the way of removing diseased parts or plants and a careful avoidance of all the conditions which seem to favor the development of the diseases are safeguards.

SPRAYING OUTFITS.

A good spraying outfit consists of a tank, holding about 200 gallons, on trucks; a pump with sufficient power, usually a gasoline engine, to supply two runs of hose; four nozzles of approved style, an extension rod to get the nozzles into the branches; and for very high trees a raised platform.

The pump.— The essentials of a good pump are that it be durable; that it work with ease; that the parts be accessible for cleaning or repairing; that it be compact; and that it have an efficient agitator.

Nozzles.— The style of nozzle depends on the work that is to be done. It is essential that the spray reach the tree in the shape of a very fine mist. A good nozzle must carry a stream for some distance and then have it break up in a mist.

Extension rods.— No matter what the nozzle it must be lifted up in the branches. For this purpose a bamboo rod lined with a $\frac{3}{8}$-inch brass or aluminum rod is best. At the lower end of the rod is a stop-cock and a hose connection — at the upper end the nozzle. Extension rods vary from 8 to 12 feet in length.

SPRAYING FORMULAS.

BORDEAUX MIXTURE.

Copper sulphate	4 pounds.
Stone lime	4 pounds.
Water	50 gallons.

Prepare a stock solution of copper sulphate by dissolving the chemical in cold water, using one gallon of water for every pound. Dissolve by suspending the copper sulphate in a burlap sack to the depth of a few inches in a barrel or wooden vessel of water until all is in solution. Place in the spray tank one gallon of this stock solution for every pound of copper sulphate to be used and fill the tank about half full of water. Prepare the required amount of lime to be used for each spray tank full by slaking the lime and adding water until a thin milk of lime is produced. Strain this into the copper sulphate solution, keeping the mixture stirred thoroly. Add water to fill the tank and apply.

LIME-SULPHUR.

Lump lime (90 per ct. or more pure)	40 pounds.
Sulphur	80 pounds.
Water	50 gallons.

Use the best grade of lump lime obtainable. Heat to boiling in the cooking vessel about fifteen gallons of water; then gradually place in it the lime. As the lime begins to slake add the sulphur. After the slaking of the lime has ceased, add enough water to make the full amount required by the formula. If fire is used, additional water will have to be added to keep the mixture to the required amount. If steam is used this will not be necessary. The mixture should be kept at a boiling point for practically one hour, or until all the lime and sulphur are combined. Constant stirring will be necessary. A measuring stick graduated according to the capacity of the vessel used will aid in keeping the volume of solution up to the required amount. When additions of water are necessary, hot water should be used. Place in an air-tight barrel when cool, and use as needed. The stock solution should test about 24° to 25° B. and at this density should be diluted at the rate of one gallon to five gallons of water for dormant spraying and one gallon to twenty-six gallons of water for summer applications. For fuller instructions consult bulletin from this Station.

COMMERCIAL LIME-SULPHUR.

Several brands of lime-sulphur, most of them of good quality, can be purchased in the markets. They are usually more satisfactory than the home-made product.

SELF-BOILED LIME-SULPHUR.

Stone lime	8 pounds.
Flowers of sulphur	8 pounds.
Water	50 gallons.

Place the lime in a barrel and pour on enough water (about two gallons) to start it slaking. Then add the sulphur and about two gallons more of water. Stir thoroly and do not let the mixture cake on the bottom of the barrel. As soon as the slaking of the lime is over, dilute at once to 50 gallons of water and strain thru a sieve of 20 meshes to the inch, working all the sulphur thru with the water.

ARSENATE OF LEAD

There are many brands of arsenate of lead of varying quality — buy the best. The amount of the arsenate to be used depends on the insect to be destroyed. Three pounds to 50 gallons of water is the usual strength but for certain beetles and their larvæ, four and sometimes five pounds are required. Arsenate of lead is the best of the arsenical poisons because it stays on longest. It may be used with either bordeaux mixture or the lime-sulphur solution.

SPRAYING SCHEDULES.

The following is a brief outline of the principal sprayings for fruits. It should be understood that diseases and insects vary greatly in destructiveness from one year to another, according to seasonal conditions. This schedule should, therefore, be used with discretion. If a disease or insect is very destructive the grower should familiarize himself with special bulletins on the subject to be had from this Station.

APPLES.

First application.— As buds show green at the tips or leaves of advanced buds are projecting about one-fourth inch, use lime-sulphur wash (32° Beaume) at winter strength, 1 gallon to 8 gallons of water, for San Jose scale and leaf blister-mite.

Second application.— Just before blossoms open, for apple scab and various leaf-eating insects, use lime-sulphur solution (32° Beaume) diluted with forty parts of water, with two to three pounds arsenate of lead to fifty gallons. May be omitted if insects are few and the weather is dry.

Third application.— When blossoms are about off, give treatment as (2). This is the most important spraying for apple scab and codling moth. Should never be omitted.

Fourth application.— About two weeks later repeat (2) if weather is favorable for apple scab; that is, moist and warm.

Fifth application.— About first week in August, if it seems necessary, repeat (2) to prevent late infections of apple scab and sooty blotch and to control second brood of codling moth.

There are a number of insects which are not effectively combated by any of the treatments in the foregoing schedule. Species that deserve specific mention are the rosy-aphis, red-bugs and leaf rollers. For literature giving detailed spraying directions to combat these pests write to the Station.

PEARS.

Spray as for apples. As foliage appears to be more susceptible to injury by the spraying mixture, dilute the concentrated lime-sulphur solution (32° B.) in the proportion of 1 to 50 for the summer spray, and apply the liquid in minimum quantities, using nozzles with fine apertures. For foliage treatments, bordeaux mixture is preferred. For special literature on pear-psylla, sinuate-borer and pear-thrips apply to this Station.

PLUMS.

First application.— For San Jose scale spray as for apples.

Second application.— When " husks " or calyces drop, spray with arsenate of lead, 3 lbs. to 50 gals. of water for the curculio. For leaf-spot on European varieties use bordeaux mixture 5-5-50 with arsenate of lead.

Third application.— For leaf-spot repeat spraying with bordeaux mixture about ten days later.

Japanese plums should be sprayed as directed for peaches.

CHERRIES.

Spray as for plums for San Jose scale and the curculio. For leaf-spot use lime-sulphur 1–50 (1) just after the petals drop; (2) 10–14 days later.

PEACHES.

First application.— For San Jose scale and leaf-curl spray late in fall or early in spring before the buds begin to swell, with lime-sulphur wash at winter strength.

Second application.— When " husks " or calyces are dropping use two pounds of arsenate of lead to fifty gallons of water for the curculio. The self-boiled lime-sulphur mixture may be used as carrier of the poison to control also brown rot and scab.

Third application.— Two or three weeks later use 8–8–50 self-boiled lime-sulphur mixture and two pounds of arsenate of lead.

Fourth application.— About one month before fruit ripens repeat treatment with self-boiled lime-sulphur mixture, omitting the poison.

INCIDENTALS.

Young orchards suffer much from sunscald.— Sunscald is brought about either by direct scalding from the sun or by alternate freezing and thawing in late winter. In either case the bark turns black, dies and is separated from the trunk. The best preventive is to grow trees with low heads. Sunscald protectors are of doubtful value.

Thinning.— Thinning fruit on the tree is practiced to obtain a larger and better product, to produce regularity in bearing, and to preserve the health of the tree. A tree should be allowed to retain only as much fruit as it can bring to commercial size. There is no loss in total quantity in thinning as greater size makes up for lessened number. Thinning is not expensive, for all fruit must be picked sooner or later and much that must be picked in the fall is not worth picking if thinning is not practiced. The time to thin is just after the " June drop."

Sterility of trees.— Varieties of several fruits often do not set crops because of the infertility of the blossoms. Pollen may be produced in abundance, seemingly perfect in appearance, and potent on the pistils of other varieties, but may fail to fertilize the ovaries of the varieties from which it comes. The remedy for self-sterility is adjacent planting. In this State the Bartlett pear is the only commonly planted tree fruit sufficiently self-sterile to need a pollinizer. Seckel, Bosc and Anjou planted every third or fourth row, insure proper pollination for the Bartlett.

Why fruit drops.— Weather conditions have much to do with the dropping of fruit. Prolonged cold prevents proper pollination, saps the vitality of the tree and causes many of the tender fruits to let go their hold upon the tree. Rain, whether a dashing shower or a prolonged drizzle at a low temperature, or even an extremely moist atmosphere without a fall of rain, weakens the chances of full development of fruits if such conditions prevail at fruit-formation. Sometimes a lack of light causes fruit to drop, and thus we may explain the greater number of fruits at the tops of trees, on well-pruned trees, in open-centered trees and in orchards not thickly planted.

Grafting old trees.— The first task in grafting is the selection of the cion. This must be of one-year-old wood. Select strong and well-matured growths, discarding all soft and pithy sticks. Cut the cion wood while the buds are dormant, pack in damp moss or similar material and store in a cool cellar until the buds break on the trees to be grafted. Care should be taken not to have the moss too wet or the cions will become water-soaked and worthless. In making the cions at grafting time the first few inches of the year's growth should be discarded, as the buds are small, poorly developed and will not start readily. The tips of the cion sticks should also be discarded.

The cleft graft is nearly always employed on large trees and can be used successfully on branches ranging from one to four inches in diameter — those less than two inches across are the most satisfactory. The limb should be sawed off squarely and be carefully split with the grafting chisel. Spread apart the split limb with the wedge on the grafting chisel and be ready for the cions. These should contain two or three buds. Beginning at either side of a bud, with two strokes of a knife, cut the lower end of the cion in the form of a wedge, one edge of which should be slightly narrower than the other. The wedge of the cion is then inserted into the cleft with its narrower edge toward the center of the stock, thus allowing the outer and thicker edge of the cion to be firmly gripped when the stock springs back against it. Care should be taken to match the inner bark of the cion with that of the stock. Heat the wax in warm water until it will work readily and then spread in a thin coat over all the cut surface including the tips of the cions and extending down the limb on both sides covering the entire length of the cleft. Make the covering air-tight or the cion will die. A good grafting wax may be made from the following formula:

GRAFTING WAX.

Resin	4 pounds.
Beeswax	2 pounds.
Tallow	1 pound.

Melt these ingredients together, cool and pour into cold water. Grease the hands and as soon as the wax becomes cool enough to handle, pull it until it assumes a brownish-yellow color. It may then be moulded into balls of convenient size, after which it should be stored in a cool place until ready for use. For a softer wax, substitute linseed oil for a part or all of the tallow.

Bridge-grafting for girdled trees.— This form of grafting is used in preserving trees injured or girdled by mice or rabbits. Ragged edges should be cleanly cut away; then make a longitudinal slit in the bark above and below the wound, and loosen the edges slightly.

The cions should be cut two or three inches longer than the space to be bridged, one side beveled off at both ends and inserted in the slits with its beveled face against the wood of the trunk. In order to guard against accidental displacement, some drive a small nail through each end of the cion taking care not to split the cion in the operation. Insert cions at intervals of about two inches around the injured surface. The ends of the cions should be covered with wax but it is not necessary to cover all of the bridged portion of the trunk.

Protection against rabbits and mice.— Clean cultivation usually prevents orchard depredation by mice, since it reduces the number of places of refuge for the animal. To a much less extent it prevents rabbit depredations. Elimination of fence rows is helpful prevention against both mice and rabbits. Trees may be effectively protected against mice by mounding the trunks with earth to a height of six or eight inches, this being the commonest and most practical protection given in New York. In making the mound, clean away grass and weeds and firm the heaped earth. The mound should be made in late fall and removed in early spring.

The wire screen cylinder is the ideal protection against cottontail rabbits. It is made of galvanized iron screening of one-quarter inch mesh. The 24-inch width should be used, cutting it crosswise with tinners' shears into sections of necessary length. These sections, if rolled over a round piece of wood may be shaped into cylinders, which, when placed about trees, are securely held by their own tension, though it is well to allow the edges to lap an inch or two. This wire screening or wire cloth can be purchased in rolls of 100 linear feet. The wire-cloth cylinder is perfect protection against mice but is more expensive than mounding.

It is not so easy to protect against the jack rabbit. With this pest, if the orchard is small, a rabbit-proof fence may be profitably put up. In most orchards, however, repellents offer the best protection. Among the many repellents recommended, undiluted lime-sulphur is far the best. A little salt increases the adhesive property of the mixture but must be boiled into the wash. Apply with a white-wash brush late in the fall and, if necessary, once, twice or oftener during the winter.

CULTURE OF FIELD BEANS.*

J. W. WELLINGTON

GENERAL REQUIREMENTS.

Climate.— The bean is eminently adapted to the climate of New York State, particularly to the counties of the central and western portion where the growing season is long enough to afford thoro ripening and yet not usually favorable to the development of fungus and insect enemies.

Soils.— Beans are often proclaimed as a crop suitable to poor soils, yet this assumption is never borne out by experience. A soil which cannot grow good wheat and clover cannot be expected to yield profitable returns. Well drained, light loams of a sandy nature are desirable. Very heavy and very light soil types are not satisfactory — the first difficult to prepare and till, the second lacking in humus and necessary food. It is generally conceded that the small-seeded varieties are better adapted to light soils than the large-seeded sorts. Limestone soils seem particularly favorable to the bean, as is shown by the location of the high-producing bean counties.

Rotation of crops.— The field bean should be grown in rotation with other farm crops — the members of the rotation depending upon the sort of crops grown in a locality. A three-year rotation often used is that of wheat, clover and beans. If potatoes and corn are included the period of rotation may be extended to four or more years, always having the bean follow the clover. Beans make an ideal intercrop for the newly-planted orchard and may be grown here in a rotation or continuously.

CULTURE.

Preparation of soil.— In planning for the bean crop it is advisable to have in mind a sod for turning under — a strong clover sod being best of all. Heavy types of soil should be plowed late in the fall or very early in the spring. Previous to actual planting the soil should be carefully harrowed and then leveled with a drag, thus leaving a smooth, even surface. During the period in which the land lies idle in the early spring frequent harrowing should be given to keep it in good tilth and maintain the water content. Needless to say that the better prepared seedbed will yield the greater crop.

Fertilizers.— Manure is a valuable fertilizer for the bean, but can be partly or even wholly replaced by turning under a green cover crop. Cooperative experiments conducted by the Cornell Experiment Station demonstrated that abundant phosphoric acid is usually particularly profitable. Strong chemical fertilizers may be more wisely used on the crop preceding the bean and, if used on the latter, should be broadcast and thoroly harrowed in. In certain seasons the fertilizer may cause injury to the germinating seed, and it is the consensus of opinion of practical growers that applying chemicals to the preceding crop is the better practice. The bean is a legume and may be expected to thrive on moderately rich soil with little or no added nitrogen. In the poorer types of soil, humus, either in the form of manure or green crops, must be added in order to secure profitable returns. Lime must be present in sufficient quantity to promote a good growth of clover.

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* Reprint of Circular No. 53, May 1, 1917.

Planting.— The bean should not be planted until all danger of frosts is past. The first week in June is early enough. If planted too early the young plants may be subjected to cold, rainy weather which will more than offset the gain of the early start. The bean is commonly sown in drills 28 to 30 inches apart. Two to three pecks of the small-seeded varieties are used per acre. Of the larger types, like the kidneys, from four to six pecks are required. Before planting a test of the germinating powers of the seed should be made. Special bean planters may be used for sowing the crop or an ordinary grain drill can be utilized. The seed should not be buried deeper than two inches except in extremely light soils.

Tillage.— Beans should be cultivated as soon as the rows show, but without covering or disturbing the little plants. Frequent shallow tillage should be given during the season of growth and if weeds are numerous they must be removed by hoes and hand. It is not advisable to work in the bean field while the leaves are damp or wet, for injuries at that time may open the way for disease.

Harvesting.— Machinery has entirely replaced expensive hand work in the bean harvest. Two rows are cut and thrown together in a windrow at one operation and then raked into heaps with a horse rake. The piles must be turned once or twice until the crop is dry, at which time the harvest is hauled to barns or sheds and stored until opportunity occurs for threshing. The bean thresher is a specially constructed machine designed on the same principle as any grain thresher but so adapted as to handle the larger seeds with little loss from crushed and split beans. Smaller plantings may be harvested and threshed by hand, but the present cost of labor would make such practice altogether out of the question with large areas.

Preparation for market.— The material as coming from the thresher is mixed with chaff, dirt and other rubbish. The chaff is removed by running the seed thru a fanning mill. Finally the beans are run in thin layers over a sorting machine from which trained eyes and hands pick the broken and decayed specimens. Caution must be observed to have the seed thoroly dry before storing and to insure this the seed should be spread in thin layers in dry rooms. The stock is then bagged and ready for sale.

Cost of production and yields.— The yield may be from five to thirty-five bushels per acre and the cost of production is in direct correlation with the yield. In most cases the cost is greater than generally supposed. According to M. C. Burritt[1] the minimum acre cost is $25, with the average between $30 and $35. The bushel cost of production varied from $1.74 one year to $3.70 another. These figures were obtained with seed at average prices. Under the abnormal and vacillating market conditions of 1917 it is difficult to make even a rough estimate of the probable profits per acre. Rainfall is one of the factors which has a very direct influence on the success of the bean crop, an extreme drought bringing almost total failure. Thus it may be seen that the prospective grower should bear in mind that the bean crop is one demanding careful thought and by no means a chance crop to fill an idle field.

BEAN TROUBLES.

Diseases: Anthracnose.— This disease is manifested in brown sunken spots which appear upon the foliage and pods. In severe attacks the leaves fall from the plant and further development of the half-grown crop ceases. The disease over-winters in the seed and care should be exercised to select good healthy looking seed stock; bordeaux mixture may be employed to hold the disease in check but as a rule spraying is too expensive an operation to be warranted.

Bacterial blight.— This disease also causes a spot upon the pods and leaves of the bean. The diseased areas are of a brown color, somewhat smaller and generally less sunken than those of anthracnose. The appearance of the two diseases is very similar and causes confusion even to the trained eye. As with anthracnose, the only remedy is the selection of healthy seed.

Disease resistance.— Several so-called anthracnose-resistant strains of field beans have recently come to public notice. Many of these have no such quality whatsoever while a few strains have considerable merit. Two supposedly anthracnose-resistant strains were grown upon the Station grounds in 1916. Both proved resistant to this disease, but one was badly attacked by bacterial blight. Before purchasing large quantities of highly advertised stock at high prices, the prospective grower should thoroly ascertain its reliability.

Insects: Common bean weevil.— This enemy of the bean causes serious damage to the industry in the United States. The small, white, footless grubs feed within the beans both in the field and storage and when mature transform into brown-gray weevils which infest the dry beans. As many as 28 weevils have been found in a single bean. Unlike the pea weevil this species unfortunately breeds in the seed and it is imperative to treat infested seed at once. Fumigation with carbon bisulphide at the rate of one ounce to 100 pounds of beans is the most efficient control measure. A tight box or barrel will make a satisfactory fumigation apparatus. The carbon bisulphide which is a volatile liquid, may be either thrown onto the beans or placed in a dish above them. The box or barrel should be sealed as tightly as possible and left for 48 hours. Extreme care should be taken to keep fire of any description distant from the operation as the gaseous form of the chemical is explosive. Carbon bisulphide may be purchased from any drug store.

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[1] In *Rural New Yorker*, 74:674. 1915.

VALUABLE VARIETIES OF BEANS.

1. **Snowflake.** Very similar to Navy Pea, differing only in being slightly earlier, less productive and having smaller seeds. Often one or more to the pod. Seed white.
2. **Navy Pea.** The ordinary pea bean. Seed white and small. Plant dwarf, 14 to 18 inches tall, with a decided running tendency, somewhat spreading, very vigorous, extremely hardy, very productive, ripens midseason.
3. **Burlinghame or Medium.** Habit of plant very similar to White Marrow, but a little later in season and more productive. Seed white and somewhat larger than the Navy Pea.
4. **White Marrow.** Plant dwarf with a running tendency, 14 to 18 inches high, very spreading, much branched, very vigorous, hardy, late, very productive. Seed of medium size.
5. **Yellow Eye.** Plant characters extremely like those of White Marrow. Seed, however, has a yellow blotch or eye on the ventral surface and a brown ring about the hilum. Seed of medium size.
6. **Vineless Marrow.** Plant quite similar to White Marrow, more erect, free from runners, later in maturity and more productive. Seed white and of medium size.
7. **White Kidney.** Plant strictly dwarf, 18 to 20 inches tall. Bushy, erect, very vigorous, very hardy, very late, productive. Seed large to very large.
8. **Red Kidney.** Seed brownish red. Plant characters same as White Kidney. Seed large to very large.

REPORT

ON

INSPECTION WORK.

W. H. Jordan, *Director.*
L. L. Van Slyke, *Chemist.*
A. W. Clark, *Associate Chemist.*
M. P. Sweeney, *Assistant Chemist.*
Otto McCreary *Assistant Chemist.*
Richard F. Keeler, *Assistant Chemist.*
William F. Walsh, *Assistant Chemist.*
[1] Arthur J. Flume, *Assistant Chemist.*
[2] Walter L. Kulp, *Assistant Chemist.*

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I. Inspection of feeding stuffs. I and II.
II. Report of analyses of commercial fertilizers.

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[1] Resigned May 20, 1917.
[2] Appointed May 21, 1917.

REPORT ON INSPECTION WORK.

INSPECTION OF FEEDING STUFFS.

[Text of this Bulletin, No. 434, May, 1917, is omitted since the data cease to have value before the Annual Report can be distributed.— W. H. Jordan, *Director*.]

INSPECTION OF FEEDING STUFFS.

[Text of this Bulletin, No. 441, November, 1917, is omitted as the data cease to have value before the Annual Report can be distributed.— W. H. Jordan, *Director*.]

REPORT OF ANALYSES OF SAMPLES OF COMMERCIAL FERTILIZERS COLLECTED BY THE COMMISSIONER OF AGRICULTURE DURING 1917.

[The text of this Bulletin, No. 440, November, 1917, is omitted as the data cease to have value before the Annual Report can be distributed.— W. H. Jordan, *Director*.]

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APPENDIX.

I. Popular Editions of Station Bulletins.

II. Periodicals Received by the Station.

III. Meteorological Records.

POPULAR BULLETIN REPRINTS.

GOAT MILK GOOD FOR BABIES.*

F. H. HALL.

Many ailing infants fed. For several years the Station maintained a herd of goats and furnished milk for many babies that were not thriving on other foods. In eighteen cases on which reports were made directly to the Station either by parents or by attending physicians, goat's milk proved helpful, and in several cases surprisingly beneficial. In a few instances use of this milk undoubtedly saved the lives of the babies, since they were losing weight under all other systems of feeding.

In only one case of the eighteen was an unfavorable report made; and of this child the physician writes: "I am sorry to report that the goat's milk did not agree much better with my patient than cow's milk. He is a child twenty months old, who does not seem able to absorb anything . . . . without developing signs and symptoms of fat poisoning."

Some favorable reports. Brief extracts from reports made in a few of the cases will show the general trend of all but the one report — from "helpful" to "a wonderful success." (No. 1) "Our baby has improved wonderfully on it. . . . We tried everything we could find for him but nothing would agree with him until we got the goat's milk." (No. 3) "Baby had been troubled with constipation and with vomiting. We started on the goat's milk . . . and conditions greatly improved." (No. 6) "The child was six months old, weighed eight pounds, and in a very scrawny condition. . . . For the sixteen weeks the child was fed goat's milk it averaged five-eighths of a pound per week gain." (No. 10) "It is a very great pleasure for me to tell you that the goat's milk which you furnished us for the past three weeks has worked wonders." (No. 15) "The results thus far are very gratifying, I assure you, for we had used almost everything, and had despaired of saving her at all." In two months before feeding the goat's milk this child had lost three-fourths of a pound in weight; in the first week of feeding she gained one-half pound, and in the next week a full pound. (No. 16) " He has been under the care of a famous baby specialist for weeks but with no results. [After beginning use of goat's milk] he began to improve at once, gaining from four to ten ounces a week." Even tho this evidence is not, in most cases, the result of the professional judgment of physicians, it is so marked that it cannot be disregarded. It apparently shows clearly that goat's milk is often a very desirable resort for infant feeding.

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* Reprint of Popular Edition of Bulletin No. 429, February, 1917; for Bulletin see p. 63.

Goat's milk used in hospital.

The Station also furnished goat milk for an extensive series of tests at St. Mary's Infant Asylum and Maternity Hospital, Buffalo, N. Y., under the supervision of Drs. Sherman and Lohnes; but the results were not so definitely favorable as the individual cases reported. The study was made, specifically, to ascertain " why goat's milk agrees better with some infants than cow's milk," the fact that it does so act being assumed. Stomach and intestinal troubles due to streptococcic infection, and removal of infants from the hospital by adoption, as well as conditions affecting institution babies generally, interfered somewhat with the success of the experiment. Of sixteen cases carried thru to a definite conclusion, it was found that goat's milk agreed better with four babies only, cow's milk better with twelve cases; but the gain was more rapid and greater in the individual cases where goat's milk was preferred than in those where the cow's milk was better. Physical and chemical examinations of the partly digested stomach contents, stools, etc., proved that the curds were smaller and more flocculent, the stomach acidity less, and the amount of undigested food remaining in the stomach after an hour's time less with goat's milk than with cow's milk. These factors help to explain the beneficial action of goat's milk in some cases of vomiting; since the goat's milk stimulates less the secretion of acid by the stomach, produces smaller curds and is more rapidly digested than the cow's milk.

It was also noted that the younger the baby the more the evidence pointed toward a greater gain on goat's milk.

Goat's milk, cow's milk and human milk.

Chemical studies made in the Station laboratory showed some marked differences between goat's milk, cow's milk and human milk, but the differences do not at present furnish an explanation of the apparently better adaptation of goat's milk, in many instances, to human feeding, when a substitute for human milk must be used.

Station goat herd.

The Station came into the possession of the herd of goats here discussed, unexpectedly, thru their presentation by Mr. H. S. Greims, of New York City. As received, the goats were a mixed lot, of various breeds and no breeds, some very good and some very poor; but after two years of weeding out, with several deaths from a rather uncommon disease known as Takosis, they were reduced in 1912 to three mature males, twenty-eight females and nine kids: One full-blood Saanen, two Schwartzenbergs and five Toggenbergs, with full-blood or half-blood descendants from the original animals, some of the crosses containing Angora and American milch-goat blood.

Food eaten by goats.

These thirty-one animals were fed experimentally from January 14, 1912, to January 13, 1913, and as careful a record as possible was kept of the feed consumed. It was impossible to secure accurate data on the expenses of maintaining the goats other than for food even under Station conditions; and it is understood that one of the reasons for relinquishing the herd by Mr. Greims was because he could not secure accurate data, as he at first hoped to do. Because of the number of the animals and their uncontrollable, wasteful eating habits it was impossible to keep a detailed account of the food eaten by each one; but so far as could be observed there was little difference between individuals in the consumption of coarse feed. About a pound a day of grain was given each mature animal. The table below gives the essential data relating to the feed eaten.

TABLE I.— AMOUNT AND COST OF FEED FOR MILCH GOATS

(Adult males, 3; adult females, 28; kids, 9.)

For Year 1912.

	Days fed.	Amount used.	Cost per 100 pounds.	Total cost.
		Lbs.		
Grain	365	14,688	$1 45	$212 96
Bean pods	199	18,180	35	63 63
Beets, mangel	46	1,550	20	3 11
Hay, mixed	273	19,560	50	97 80
Grass	122	24 300	15	36 45
Pasture	132	....	....	28 00
Total	....	....	....	$441 95

The monthly cost of food varied considerably with the season, averaging about one dollar apiece from September thru January, and from 80 cents to 96 cents for the other months, the lowest figure being for July.

Milk yield.

For three years accurate records were kept of the milk production of individual does, their breed and milk production being as follows:

TABLE II.— MILK YIELD OF MILCH GOATS.

	MILK YIELD		
	1910 *Lbs.*	1911 *Lbs.*	1912 *Lbs.*
No. 5. Full blood Schwartzenberg	678.3	913.3	600.7
No. 6. “ “ Short Haired Toggenberg	778.4	1,455.7	377.1
No. 7. “ “ “ “ “	186.5	763.5	870.7
No. 8. “ “ “ “ “	1,189	838.3	*
No. 9. “ “ Long “ “	210.3	1,167.1	705.5
No. 10. “ “ Short “ “	†	1,361.4	869.2
No. 11. “ “ Saanen	1,028.5	1,845.2	1,391.1
No. 12. “ “ Schwartzenberg	120.5	613	424
No. 15. Toggenberg-Angora	496.5	801.3	725.5
No. 16. “ “	457	899.4	655.9
No. 17. “ “	....	211	63.9
1910 kid of No. 5	....	846	861
Toggenberg-American	....	406.5	472
1911 kid of No. 5	....	....	741.2
1911 kid of No. 5's 1910 kid	....	....	‡
1911 “ “ “ 6	....	....	428.1
1911 “ “ “ 10	....	....	528.1
1911 “ “ “ 12	....	....	583.2
1911 “ “ “ 16	....	....	336.8
Schwartzenberg-Toggenberg	....	....	409.9
Toggenberg-Saanen	....	....	439.1
“ “	....	....	413.7
“ American	....	....	489.6
“ “	....	....	518.8
Saanen-Angora	....	....	301.7

It is to be noted that during 1911, the production of four of these animals, Nos. 6, 9, 10 and 11, was very satisfactory. With only one animal did this standard of production continue during 1912, namely, No. 11. This pure bred Saanen appears to have been a somewhat unusual animal, as her milk yield in 1911 was 1845.2 pounds. During 1911 and 1912 several of the animals gave from 700 to 900 pounds of milk, which is perhaps as good a yield as may be expected, excepting from animals considerably above the average. It should be noted that the period of lactation is considerably shorter than with cows, continuing from 250 to 300 days with most of the does.

Cost of food and milk.

The average cost of food per goat for the year was $11.05, making the cost per day three cents. If it was possible to get any number of goats together like No. 11, the Saanen, the keeping of goats would be a profitable proposition for a family supply. On the basis of a food cost of eleven dollars a year, her milk, during the year of best production, would cost 1.27 cents a quart, the average of the three years being 1.65 cents a quart. The best Toggenberg one year produced milk for 1.62 cents a quart, but her average for the three years was 2.70 cents a quart. The average food cost of the milk, for 1912, of all the goats was 4 cents a quart, and the average cost for the three years was 3.4 cents a quart. The average fat content of the milk was 3.71 per ct., of total solids 11.76 per ct. The average food cost of a quart of milk with the Station herd of 25 Jerseys during the past three years has been .92 cents per quart; the average fat content 5.9 per ct., and total solids 15.20 per ct. This shows the cows to be cheaper producers of milk and milk solids than the goats under the conditions prevailing at this Station.

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* Would not breed. † Did not breed. ‡ No milk.

Composition of goat's milk.

Daily analyses were made of the mixed milk of the goats for seven weeks in May and June, 1912. These showed a milk of quite uniform composition for the herd as a whole, since the fat varied only from 3.2 per ct. to 4.4 per ct., with a marked tendency to remain about 3.5 to 3.8 per ct. Individual goats, however, varied greatly from these figures, as shown by analyses on three successive days of the milk of eleven of the does. In these animals the fat ranged from 6.0, 7.8, 8.4 per ct. for the tests of one animal to 3.0, 2.2, and 1.8 for those of another. The solids-not-fat ranged for the herd from 7.7 per ct. to 8.6 per ct., with a general range between 7.8 per ct. and 8.2 per ct. and extreme variations with separate individuals from 7.0 per ct. to 10.2 per ct.

Conclusions.

As a general summary of Station experience with goats it would seem that only in exceptional cases where the use of otherwise wasted feed would reduce the cost of keeping, or with exceptional animals, like No. 11, could goats be expected to produce milk as economically as cows. For family use, however, in places where it is impossible to keep a cow and where a goat or two could be kept, these animals might prove valuable aids in maintaining babies or small children in good health. The milk is palatable, nutritious and easily digested, very helpful in certain cases of poor nutrition, without odor when drawn under proper conditions and with proper care, and practically free from the liability to transmit certain diseases, like tuberculosis, which may be transferred to children from cows.

The odor from goats is decidedly unpleasant at times, but as this is mainly due to the male, the annoyance from this source may be reduced to a minimum where a few does only are kept under proper conditions.

EUROPEAN GRAPES SUCCEED IN NEW YORK.*

F. H. HALL.

Choicest of table grapes.

The translucent, white Malagas and the bright Tokays — favorites of Thanksgiving and Christmas dinner tables — represent a class of grapes long believed impossible to grow outdoors in the East. Early settlers in eastern America were thrilled by the sight of grapes of many colors and types growing wild in the woods in surprising abundance; and many of them spoke, in letters home and in reports, of the splendid prospect these fruits offered of limitless feasting and drinking, especially the latter; for it was as a source of wine that the grape then made its strongest appeal. It was soon found, however, that neither for wine-making nor for the table did the native grapes equal the kinds the pioneers had known at home — the numerous and widely varied types of *Vitis vinifera*. Accordingly, almost from the first settlement of America, attempts were made to introduce into the New World the grapes of the home-lands.

Without exception, these attempts were failures. The plants the winters did not kill the phylloxera took, and if, by any lucky chance, fruit was produced, mildew and black rot made it a mass of corruption. These untoward conditions, pests and diseases ruined every vineyard of European grapes in the East, sooner or later, even though several of the attempts to establish such vineyards were backed by abundant capital and managed by expert grape-growers from France or Switzerland.

So, for three-quarters of a century, now, it has been thought out of the question to grow European grapes — wine or raisin grapes — in the open east of the Rocky Mountains; though they have long been a main standby of California's horticulture. We of the East, unless privileged to share the product of some hothouse-grown vine, have been able to enjoy these delicious fruits only after they have made long journeys across the Atlantic or across our Continent.

In one way this has been fortunate; for the inability to introduce the Old World grapes has led to a wonderful development of the native species, until we have hundreds of attractive and delicious grapes, equalling if not excelling the European grapes in some respects, and introducing some qualities all their own.

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* Reprint of Popular Edition of Bulletin No. 432, April, 1917; for Bulletin see p. 561.

Yet the best varieties of *Vitis vinifera* have a sweetness and a richness of flavor which appeal strongly to most tastes, an absence of the " foxiness " that adapts them to making the choicest of wines and champagnes, and a meatiness and solidity that keeps them edible long after the season of native grapes and that makes it easy to turn them into raisins.

These qualities have so appealed to those who could afford the expense that many European grapes have been grown in forcing houses in order that the owners might enjoy these grapes in their perfection. Grape breeders have availed themselves of the presence of these hot-house vines to cross the European varieties on the native kinds; and many of our best table grapes owe much of their fine flavor to the strain of *Vitis vinifera* blood introduced thru plants that would have failed in the open.

Failure now preventable.

Recent progress in grape culture proves that the old failures with European grapes were due to causes quite easily controlled; and we know, now, that many varieties of *Vitis vinifera* — though not all, by any means — may be grown and ripened successfully in New York State. It is improbable that we can ever grow Malagas and Tokays to perfection in the open; for these are varieties requiring a very long, warm season; but dozens of other varieties, many equalling these familiar ones in beauty, flavor, and size of bunch and berry, are promising candidates for trial in New York, particularly in those sections where the late-season native varieties do well.

Winter-killing, perhaps the most common cause of failure in the past, can be easily prevented by covering the vines with a few inches of earth, a simple, rapid and inexpensive operation, with vines properly pruned for the purpose. Phylloxera — the root-sucking plant-lice that have been so destructive to European grapes in California and in Europe — do little harm to roots of certain American species of the vine, and Vinifera cions unite finely with stocks of these native species. Mildew and black-rot are no longer dreaded by the vineyardist who knows the merits of bordeaux mixture and similar fungicides.

European grapes do well at Geneva.

As proof that these measures are successful in the growth of European grapes in the East the Station can cite long and extensive experience. A few vines of pure *Vitis vinifera* varieties have been growing on our grounds for nearly fifteen years, and ninety varieties for more than five years. During the life of the last collection of vines the weather conditions have at times been among the most severe ever known, both cold of winter and heat of summer, excessive rainfall and long, severe droughts having tested the endurance of the vines to the limit. A few have succumbed, but the great majority have come thru in as good shape as would a similar assortment of native varieties. From these last vines, grafted in the spring of 1911 upon stocks of native seedlings in place in the vineyard, some grapes were harvested in 1912, and since that time they have, as a whole, yielded as well or better than most of our native grapes. Neither soil nor climatic conditions at the Station are favorable for grapes of any kind, as Catawba ripens with us but rarely; yet some of these *Vitis vinifera* vines have matured heavy yields of splendid grapes, admired of all who have been privileged to taste or see them; while other kinds have shown less beautiful fruits but quantities of berries that give much juice of unexcelled quality for the making of either unfermented or fermented drinks.

Necessary points in culture.

The principal difficulty at present in introducing these new sorts to New York viticulture is the difficulty of obtaining the plants. Few eastern nurserymen catalog varieties of *Vitis vinifera*, and the supply in California of varieties that will succeed in New York and are rooted on native phylloxera-resistant stocks is small. This makes it almost essential to graft stock at home, which can be easily done provided cions are obtainable, by one who understands bench grafting; but the simplest and best plan is probably to graft new Vinifera tops on native stocks in place. These may be set six by six, since the Vinifera vine requires less room than native varieties. The ordinary cleft graft succeeds well on the grape, though it may be necessary to saw, rather than to split, the cleft if the grain of the vine twists. The top of the native vine should be so cut off that the cleft will be at the surface of the ground. By another method, native and Vinifera plants are set side by side in the vineyard, grown a year on their own roots, then united by cutting away portions of each vine and binding together the exposed surfaces until they unite. The following spring the Vinifera top is severed from its roots and the native top pruned away. The Station has succeeded satisfactorily in uniting Vinifera cions to Riparia stocks set in the nursery row by whip-grafting two weeks after the rooted cuttings were placed.

As winter protection is necessary provision must be made for replacing the old trunk, when it becomes too stiff to bend down easily, by a new one. A replacing spur, continued by one cane each year, should be left near the ground level, on the main trunk. This main trunk is carried only to the lower wire of the trellis. At the winter pruning, two one-year canes are selected, to be tied along this wire, one on each side, and two renewal spurs are left as close to the top of the main trunk as possible. The young shoots which spring from the one-year canes, instead of trailing to the ground or running out along the trellis wires, grow erect. Advantage is taken of this in the pruning system adopted at the Station. The canes and renewal spurs as described above are tied along the lower wire, then the young shoots which come from these grow upward to the second wire. When they are four to six inches above this they are pinched off just above the wire and any which have not already fastened themselves are tied to prevent the wind breaking them off. At the same time if any of the axial buds on the shoots — buds which form at the base of a leaf petiole and which usually remain dormant until the following year but which sometimes start into growth on the younger shoots — have begun to form secondary shoots, they are rubbed off, beginning with the node next above the upper cluster and going down to the old cane. This gives the clusters more room and better light. Soon after the first heading-back, the upper buds of the young shoot start lateral growth. These secondary branches usually grow upright and when they are several inches high they are topped off with a sickle. This heading-back results in stockier and more mature canes for the following year and if properly done adds to the fruitfulness of the vine and matures the fruit better.

For winter protection, as soon as there is danger of the ground freezing, the vines are pruned, cut from the wire and bent to the ground. Some care has to be exercised in this that the trunk is not broken. While one man holds the vine down two others throw on dirt from each side till it is covered to a depth of five or six inches. During the winter this dirt will settle down considerably and sometimes part of the trunk will be uncovered but no injury has been noted in such cases. This work can be done so rapidly that it adds but a few cents to the cost of growing the vines.

Value for New York.

The Vinifera grape should make a most welcome addition to the home-gardens in New York. The rich, clear flavor, the high quality and the size and appearance of many of the kinds of this species adapt them splendidly to the home-plantations where quality should be the chief consideration. For the amateur, the lover of good fruit, these can be recommended unhesitatingly. On the other hand, it would not be wise to attempt growing them on a large scale commercially until we have had more experience with them and wider knowledge of the requirements of this species, though the commercial grower who is supplying a high-class local market where good quality counts could well afford to begin testing some of the best varieties to find which are suited to his conditions and to the demands of his market.

The value of the wine varieties in the State is problematic. It will take several years to test out these varieties to find which are best adapted to the conditions in the wine region of the State and, after the proper kinds are found, it will be several years longer before sufficient vines could be brought into bearing to have any effect upon the wine industry. It is a question whether the demand for such grapes would be great enough to justify the expenditure of the time and money necessary to develop them. There are, however, some very good wine sorts which, at the same time, make desirable table grapes and it would be well worth while to begin to test the best of these in the Keuka region.

For nearly a hundred years breeders of grapes have been blending this foreign species with our native kinds and, as a result, of all the grapes which we mark as high in quality, practically every one has some Vinifera blood in it. Altho this hybridization has been going on for nearly a century the work has been desultory and not much more than a dozen European varieties have been used as parents and these chiefly the late-season, greenhouse sorts. There is no other field of fruit-breeding which offers greater promise than the continuation of this work and it is no idle speculation to say that viniculture in New York will be greatly enriched by the Vinifera hybrids which will be introduced in the next fifty years. This Station is already growing several hundred such hybrids.

Varieties. Since the Vinifera grape varieties have originated in localities with longer, warmer seasons than those at Geneva, only a small proportion of these varieties can be expected regularly to ripen here; but many other sorts will undoubtedly do so in sections of the State where the later native varieties succeed. A few varieties that promise success on almost any good grape soil are Bakator, Golden Chasselas, Chasselas Rose, Lignan Blanc and, for wine and table, Blue Portuguese.

Many other varieties are described in the regular edition of this bulletin, and classified with reference to their general adaptability, suitability for testing in favorable grape sections or lack of promise for New York. In each of the first two groups, varieties suitable for the table and for wine making are listed separately.

WHY AND WHEN WINTER KILLS GRAPES.*

F. H. HALL.

"For every evil under the sun,
There is a remedy, or none;
If there be one, strive to find it,
If there be none, never mind it."

Necessity for investigation.

The winter killing of fruits is an evil for which a remedy would be heartily welcomed; but effective control of winter's rigors or prevention of their dire consequences has seemed to many investigators so "impossible" as hardly to be worth study. Recent experiments with Vinifera grapes at the Station have shown, however, that winter injury, one of the great obstacles to success with these grapes in early attempts to grow them, can be easily controlled. Can like effective, practicable methods of preventing the occasional disastrous freezing of fruit buds or fruiting canes of American grapes be developed? As experience has often shown, the best "striving" for a remedy for any evil is a thoro investigation of the conditions under which it develops and of the causes which produce it. The occurrence of three years of marked shortage of crops in the "Grape Belt," due to winter injury, has given opportunity for such study of the factors influencing winter killing; and the results of these investigations are presented in detail in the bulletin of which this "Popular Edition" is a condensation.

Years of grape shortage.

The grape crop of 1910 in the section about Fredonia, particularly in the Station vineyard there, showed that more than half of the fruit buds were killed during the previous winter; again in 1913, the yield dropped to about half what it was in 1912 or 1914; and in 1916 the tonnage was the lowest known for many years.

Low temperature alone not responsible.

While each of these low-yield crops was preceded by notably cold weather during the preceding winter, it was not low temperatures alone that determined the injury, nor was the extent of injury in the three seasons measured by the fall of the mercury. The half crop of 1910 followed a winter when the lowest reading of the thermometer was —10° (in February); yet the crop of 1912 was practically uninjured by the winter altho the mercury dropped to —19° on February 10th of that year. Similarly, the crops of 1914, preceded by a thermometer reading of —15° in February, was twice as large as that of 1913, when the lowest temperature recorded for the winter was only 0° or slightly below. The abnormally low temperature of —16° in mid-March of 1916 would indoubtedly have caused a poor crop in the fall of that year, but other factors were necessary to bring the yield down to one-third of what the vineyard had produced.

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* Reprint of Popular Edition of Bulletin No. 433, April, 1917; for Bulletin see p. 586.

[Plates LV and LVI illustrated this text]

Lack of maturity leads to dead buds.

Undoubtedly the most important factor in winter injury to grapes is immaturity of tissues in the fall. Preceding the severe injury in the winter of 1909–10 was a marked shortening of the season of growth, so that fruiting wood and buds went into the dormant condition soft and moist. On October 12 and 13, toward the close of the harvest of 1909 and while many grapes were still on the vines, a freeze stopped growth so that the tissues had no further opportunity to ripen and harden. Of course, the drop to 27° at this time did not actually kill buds or wood on which the next season's crop depended; but their immaturity allowed them to succumb easily at one or more of three critical times during the winter. These were a long-continued period of cold weather in December with 5° as a minimum, a sudden drop to —10° in late February, and a freeze in late April (27°) following an abnormally warm period when buds probably started growth.

In 1912 very heavy rainfall in September with temperatures far above the normal promoted succulent growth and again led to immature wood and improperly ripened buds. In pruning during this fall it was impossible to find enough well-matured canes on two-year wood to provide for a full setting of fruit, and it was necessary to use some canes coming from older wood. Only three-fourths as much wood was pruned away this fall as in the previous year, owing to the light weight of the withered, immature canes, yet fewer buds were left for fruiting than in that year. Altho no remarkably low temperatures were recorded for the winter of 1912–13 the poorly-matured canes and buds again suffered, so that the crop was only a little larger than that of 1910.

In the late summer of 1915 low temperatures and cloudy weather made growth of fruit and wood slow; and again a warm, rainy September and a cool, dark October left wood soft and succulent, so that it was in no condition to resist the low temperatures of February (—13°) and March (—16°) that followed.

Some winter killing overlooked.

Frequently light crops follow heavy ones in vineyards, the common assumption being that the heavy crop exhausts the vine's supply of food, which it must have an "off year" to replenish. This is undoubtedly true to some extent, but winter killing of part of the buds also enters here as a factor. The energies of the vine are probably devoted mainly to ripening the fruit as long as this remains immature on the plant, leaving the final maturing of buds and canes to the comparatively short period which comes between the grape harvest and stoppage of growth by cold. With a large crop, too little strength remains in the vine to mature the buds properly, and many of them are killed or changed in character by winter without general death of the canes.

Grape buds compound.

The bud of the grape is compound, consisting frequently of three buds enclosed within the same bud scales: The first, or primary, to produce fruit; a secondary which ordinarily produces wood only, but may sometimes bear fruit; and a tertiary, which ordinarily remains undeveloped, but expands as a shoot in case the others are destroyed. The unnoticed winter killing may cause the primary bud to fail, when the secondary, or even the tertiary bud, puts forth a shoot; and the owner of the vineyard thinks the vine did not produce a fruit bud at all, because of food exhaustion. Many instances of this character have been observed in the studies at Fredonia; and, on the contrary, it has also been noted that two full crops may succeed each other, like those of 1911 and 1912, when winter killing did not harm the second crop because conditions allowed proper maturity of both fruit and wood in 1911.

Varieties differ in resistance to cold.

The winter killing of the season of 1915–16 showed that varieties differ greatly in their resistance to winter injury. The destruction of buds in the Station vineyard at Fredonia varied from 10 per ct. to 100 per ct., or complete killing. This vineyard contained about 150 varieties or representatives of species; and of these twenty lost more than 80 per ct. of their buds, and fifteen of them lost less than 20 per ct.

These varietal differences indicate, to some extent, that hardiness to winter injury is a species characteristic, though species are so thoroughly intermingled in many varieties that very definite conclusions are not warranted. Labrusca influence definitely lessens the tendency to such harm; while varieties with Vinifera blood, in any combination, usually show a high percentage of winter injury. Aestivalis varieties appear slightly less affected, and Riparia still less but more than the relatively hardy Labrusca crosses.

This specific difference in groups of varieties may be associated with greater or less hardness of wood, since grapes are known to differ considerably in this respect, but studies have not been carried far enough to establish this correlation.

Index to maturity.

In nearly all the cases studied, immaturity of wood and buds for the season was indicated by immaturity of the fruit, as shown by high acidity and low content of solids, especially sugar. The poor crop of 1910 followed that of 1909 with less than 16 per ct. of sugar, that of 1913 was prophesied by a sugar content of only 14 1/6 per ct. in the crop of 1912, and that of 1916 by 1915's sour fruit with only 14½ per ct. of sugar. The good yields of 1911, 1912, 1914 and 1915 were preceded by crops with sugar approaching 17½ or 18 per ct.

This index cannot be taken as infallible, however, since many other factors than maturity of wood and bud may influence the degree of winter killing. Most perfect maturity may be followed by winter injury, if warm and cold periods alternate too violently or too frequently; but such maturity undoubtedly serves as a protection in ordinary winter weather.

Other factors considered.

Since immaturity predisposes to winter injury, those factors which tend to produce slow growth or to prolong unduly the season of growth are "accessories before the fact" of winter killing. Among the factors found to exert such an influence, improper drainage was perhaps most injurious. In some instances, severe pruning after damage by late frosts in the spring induced rank growth of wood, which did not properly mature and therefore suffered during the following winter.

Excellent opportunity was given to test the effect of fertilizer elements on maturity, since the Station vineyard contains duplicate plats fertilized with these elements alone and in combination with each other and with lime. None of the elements — nitrogen, phosphorus and potash — influenced maturity; so none of them can be considered as having any relation to winter injury of grapes.

Conclusions.

Winter injury in very unfavorable seasons cannot be prevented, of course; but the evidence indicates that something can be done to lessen the amount of damage in such years and to reduce it to a minimum in other years.

First, vineyards should not be located on land that cannot be readily drained; and drainage systems should be installed or improved, where possible, in vineyards already established.

Second, the time of discontinuance of vineyard cultivation in mid-summer or later should be governed by the weather, allowing cover crops or weeds to grow longer if soil is full of moisture, so that the transpiration of these plants may lessen water in soil and check undue luxuriance of the grape vines. Wide-leaved cover-crops, or green-manuring crops, like rape and cowhorn turnips, may shade the ground so much, however, that the check of evaporation from the soil surface is more detrimental than the transpiration from the plants is helpful. Thin seeding is the remedy where such conditions are anticipated.

Narrow-leaved plants like the grains, or sparse-foliaged crops like buckwheat, exert comparatively little shading effect.

Slowly available forms of nitrogen, such as raw bone, leather scrap, hair, etc., should be avoided unless used very early, so that their stimulus to growth may not come so late that the wood is left immature.

POOR VENTILATION INJURES STORED POTATOES.*

F. H. HALL.

Blackheart not always due to overheating.

A few years ago a diseased or abnormal condition of shipped potatoes was found to be due to overheating during a delay in transit. The centers of the potatoes became deep brown or black, so the name " blackheart " was applied to the trouble. Bartholomew, the investigator of this condition, found that, to avoid danger of frost when the shipped potatoes were delayed, the car had been heated excessively; and he was subsequently able to reproduce the blackening of the potato flesh by subjecting the tubers to temperatures from 100° to 113° F. He gave, as a secondary cause that might produce or intensify the trouble, lack of oxygen from insufficient ventilation.

It was accidentally found at this Station that potatoes allowed to receive only small quantities of air showed the same appearance, tho exposed only to temperatures far below those mentioned by Bartholomew. Extensive and long continued series of experiments begun by the Station botanists in consequence of this discovery have proved that poor ventilation, even when temperature conditions are normal, may lead to serious injury from blackheart. This fact has a very important bearing on the problem of proper storage for potatoes.

Experiments with tubers in jars.

In the earlier Station experiments jars were used. Weighed quantities of clean, dry tubers were placed in the jars with known proportions of air and the jars hermetically sealed. Different lots of these were kept for varied periods of time at temperatures within and above the range of those occurring in storage, extending to 70° F.

These tests proved conclusively that potatoes can not long stand close confinement with limited quantities of air. The lower the temperature and the greater the proportion of air, the longer would the injury be averted or the less serious would it be; but unless allowed nearly twenty times their volume of air the tubers thus hermetically sealed would not sprout normally in the jars and were liable to show typical blackheart.

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* Reprint of Popular Edition of Bulletin No. 436, July, 1917; for Bulletin see p. 277.] [Plates X, XI, XVII and XVIII illustrated this text.]

With equal volumes of tubers and air, confinement for ten or twelve days at a temperature of 70° caused the tubers in the jars to become moist over some or all of their surfaces. When the potatoes are removed from the jars, these moist areas turn brown, and the flesh at the center of the tuber changes to pink and then to black — blackheart. Reduction of the temperature at which the jars were held to 55° or 60° F. delayed the appearance of the moist areas and the accompanying development of blackheart to 20 days; and tubers held at 40° remain uninjured for from 23 to 40 days. When the jars are only one-half or one-quarter full of potatoes, thus increasing the proportion of air, the development of the injury is delayed; but it appears before the normal length of time for storing potatoes is reached.

Until ten times as great a volume of air as of tubers was enclosed in the jars, little or no sprouting of the potatoes took place; but with about nineteen times as much air the tubers sprouted normally and showed no other signs of injury.

Tubers stored in tanks.

As these jar tests indicate that lack of air soon results in injury to tubers or their death, it was evident that improper storage conditions might lead to the same trouble. Accordingly, many tests were made with potatoes stored in tall, slender, upright metal tanks, closed at the bottom but usually open at the top. Each of the tanks was nine inches in diameter, but they were made of various heights, ranging from 6½ to 10½ feet, and wire-netting separators were placed in the tanks as they were filled with tubers thus dividing them into sections so that the effect of the various conditions imposed could be easily determined for the potatoes at different depths. Altho these tanks, with sides impervious to air, do not imitate exactly the conditions in piles or bins of potatoes of different depth, it is believed that they approximate them so closely that the results are a good guide to proper storage conditions.

Seven experiments were carried thru, using from one to four tanks, and in some cases sealed or unsealed jars were also used as checks or guides. In all the tests the conditions outside the tanks were quite like those actually surrounding stored potatoes, and in one case the tanks were placed in a cellar used for storage of apples, potatoes and roots. Careful records were kept of temperatures and only slight differences in readings were found between thermometers surrounded by the potatoes and others placed at corresponding heights outside the tanks.

The results secured in the different tanks and different tests do not show strict uniformity and there were noticeable differences between individual tubers under the same conditions in each tank; yet the very plain teaching from the tests is that it is **not safe to pile potatoes more than six feet deep,** if they are to be held for some months, even if the temperature remains below 40° F. If to be stored more than three or four weeks where temperatures rise above 50° the tubers should not be piled more than three feet deep.

The injury to the potatoes in the tanks is very similar to that of the tubers in the sealed jars, often with the addition of rotten spots due to the action of fungi and bacteria. In general, tho, they show the same collection of moisture on the surface, external discoloration when exposed to the air and frequent occurrence of typical blackheart. Like the potatoes in jars those in tanks failed to sprout normally when held long at any considerable depth even if the temperature favored germination.

Potatoes in " pits."

After these studies were begun it was learned that potatoes stored in " pits " (i. e., piled in conical heaps on the ground and covered with coarse manure, stalks, straw, etc., and soil), sometimes showed blackheart; so a study was made of three such pits. No injury to the potatoes resulted altho conditions were apparently quite favorable to occurrence of the trouble. This would indicate that ventilation in " pits " is better than would be supposed from the conditions.

Cause and appearance of blackheart.

There is no doubt that blackheart may be caused, as Bartholomew states, by overheating potatoes, especially if poorly ventilated, but it is equally clear that lack of air alone produces an almost identical condition at quite low temperatures. That it is to lack of air, or oxygen, not to the accumulation of carbonic acid gas which the potatoes exhale, that the trouble is due is proved by two tests. In these tests the potatoes were enclosed in sealed receptacles and chemicals were placed below the tubers, but not in contact with them, to absorb this gas as it was produced. In spite of the withdrawal of this possibly harmful gas the tubers developed blackheart as markedly as those under similar conditions but with the gas present.

Blackheart produced by heating and that caused by lack of oxygen are very similar, so far as the flesh of the tuber is concerned; but the skin of heat-blackhearted potatoes shows no signs of injury, while those affected with blackheart from the lack of oxygen are more or less browned or discolored.

In both types of the injury the flesh first turns pink, then brown and finally black, and in extreme cases the discolored portion may become tough and leathery and so shrink that considerable cavities are formed.

Blackheart potatoes for seed. The production of blackheart by lack of air is accompanied by a loss of vitality of the eyes, so that such blackhearted potatoes will not sprout normally, putting out very small, short stems or none at all. These sprouts, however, are not like those in "spindling sprout" — long, and very slender — but are merely dwarfed or miniature sprouts. Unless the trouble is so serious that the surface of the tubers is discolored, the sprouting ability of the potatoes is not greatly affected. Sprouting may be delayed by the small supply of oxygen while the tubers are in storage, but on free access of air normal sprouting occurs unless the internal injury has gone so far that evidence of it shows on the surface.

With heat-blackhearted tubers, decay usually follows severe injury, and germination is not greatly affected unless the internal injury is severe; so the same rule applies: If potatoes in the same lot with those showing blackheart do not present some external evidence of the trouble, either discoloration or decay, they may probably be safely used for seed. In cutting the seed it may be well to reject those tubers showing very extensive areas of blackened flesh.

Application of results to potato storage. The results of this investigation emphasize the importance of providing ventilation for potatoes in storage. The need of ventilation depends very largely upon the temperature. As the temperature rises the volume of air required increases rapidly. At low temperatures, potatoes may be stored in deep piles for long periods of time. At high temperatures, it is necessary to avoid deep piling or else provide special means of ventilation. If the temperature is kept below 40° F. potatoes may be piled in bins and cellars to a depth of six feet without any ventilation except that provided thru free access to the air overhead. Under such conditions, potatoes may be stored with safety for at least six months and perhaps longer. It is probable that no harm will result if the temperature goes up to 45° F. for a few days. But a long period of storage followed by a two weeks' exposure to a temperature of 50° F. or higher is liable to result in the ruin of most of the tubers below a depth of about three feet. Potatoes stored in deep piles should be carefully watched in the spring as the temperature rises. A few days of high temperature may cause much loss.

It is not in the province of this bulletin to discuss the means by which the ventilation of potatoes may be secured. The methods to be employed will vary according to circumstances. The principles to be kept in mind are as follows:

(1) Potatoes stored at high temperatures require more ventilation than those stored at low temperatures.

(2) Better ventilation is required for potatoes which are to be stored for a long period than for those which are to be stored for only a short time.

(3) Until more accurate determinations are made, six feet should be regarded as the maximum depth to which potatoes may be piled without special provision for ventilation when stored for six months at temperatures below 45° F. If greater depths than six feet are employed ventilators should be provided and so arranged that none of the tubers will be more than six feet distant from an abundant supply of air.

(4) At temperatures of 50–70° F. potatoes should not be piled over three feet deep if they are to be kept longer than about three weeks.

(5) No kind of ventilation is sufficient to prevent the occurrence of blackheart in potatoes kept for even a few days continuously at a temperature above 100° F.

(6) Complete exclusion of the air will ruin potatoes at any temperature.

(7) Small potato pits do not need ventilation; but some provision should be made for the ventilation of large pits.

ONION NECK-ROT IN STORAGE HOUSES.*

F. H. HALL.

Old, but not well-studied disease.

In the fall of 1913 word was received at the Station of an outbreak of disease in an onion-storage house in western New York. The disease was said to threaten the entire stock of onions in the house. Examination proved the trouble a serious one, of which the exact cause was unknown, but which had previously been reported from many places under various names, as mold, gray mold, storage rot, onion rot, onion bulb rot, dry rot, black-neck rot, dry neck-rot, stem rot and neck-rot. As neck-rot is simple and appropriate it has been chosen as the common name for the disease.

Trouble of fungus origin.

Only casual study was needed to prove the trouble due to a fungus, and to identify the organism causing it as one of a large group of disease-producing fungi. The disease, or others similar to it in some respects, had been ascribed by plant pathologists to different members of the genus Botrytis, or to species of allied genera; but careful study of the fungus and comparison of it, point by point, with more than a dozen other species reported as causing onion rots of this type or known to affect other species of the onion family, proved it a new species and resulted in giving it the name of *Botrytis allii*.

Nature and extent of the disease.

The disease is most common on stored onions, on which it usually appears, externally, as large black bodies or crusts, generally on the bulb near or surrounding the neck, less commonly on the sides, and occasionally at the base. Beneath these bodies or crusts one or more of the scales, or the thickened leaf bases that make up the bulb, are rotted — but without the production of much moisture, thus leading to the name "dry rot." On the onions the disease may also show as a dense ring or band of closely interwoven threads covered with smoke-gray fruit-bodies of the fungus, hence the name "gray mold." Occasionally, an onion attacked by this fungus will be found softened all thru, moist, and with a very offensive odor; but such rotting is believed to be due to bacteria which follow the true neck-rot fungus.

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* Reprint of Popular Edition of Bulletin No. 437, July, 1917; for Bulletin see p. 319. [Plates XXIII and XXIV illustrated this text.]

In some outbreaks, as in the one which led to the investigation, the loss of the stored onions from this disease may be almost complete in many houses, especially those poorly ventilated. This was the case in western New York in the winter of 1912–'13. Again in 1915–'16, following a growing season very unfavorable for onions, storage losses were enormous. In some Michigan storage houses where the disease was also studied, the loss from neck-rot early in the winter of 1915 averaged one crate out of every six stored; while by January 4, the losses were over 50 per ct. of the total stored crop. One grower who put in storage 2200 bushels was later obliged to dump 1200 bushels of these on the fields because of the neck-rot. Of the entire crop only two bushels were fit to save for seed bulbs. Ordinarily the neck-rot injury appears only as one factor in the " shrinkage " of stored onions, the others being smut fungus, onion maggots and sprouting of the bulbs. In several instances neck-rot was responsible for more than 60 per ct. of the shrinkage, and was undoubtedly the cause of part of the sprouting, also, since the attacks of the fungus released some moisture and induced premature growth.

The neck-rot fungus may attack the growing crop onions in the field at almost any stage. Even early in the season it may produce white, circular or elongated spots on the leaves, surrounded by areas with a yellowish or water-soaked appearance. Later, the fungus, going down thru necks and bulbs or attacking thru the roots and working upward, checks the growth of the bulbs and thereby causes a large proportion of " seconds " or " picklers " thus reducing the yield and lowering the value of the crop; or else the fungus in the bulbs may pass unnoticed into the storage house, where, under favorable conditions, severe rotting may take place. On the seed crop the attacks of the fungus, which usually accompany a period of excessive moisture, cause a failure of the seed heads to produce seed — a condition almost identical with the so-called " blast " of the seed crop, thought to be due to either extreme dry or wet weather. Such failures to secure seed in New York in 1913 and in Michigan in 1915 are believed to have been due largely to attacks of this fungus.

How the disease starts.

Like other fungus diseases this one is distributed mainly by spores (minute bodies which correspond to the seeds of higher plants). These may be carried by the wind to the onion plants or remain in the soil and infect the bulbs thru the roots. The fungus does not pass readily from a diseased bulb to a healthy one in storage because of the resistance offered by the one or more parchment-like dry scales which cover the onion; but where this dry covering is broken or removed and the skin of the fresh, succulent bulb-scale beneath is ruptured, as by a cut or bruise, the bulb may easily become infected. When healthy onions are in close contact with a mass of wet, sprouting and rotting specimens, or when, for any reason, the outer, papery covering and the scale beneath it are kept moist for some time, even tho not in direct contact with the rotting bulbs, the disease may spread to them, since the presence of moisture causes germination of the spores and softens the tissues so that the fungus finds an entrance. A spore in a single drop of water that evaporates slowly may be all that is needed for a center of infection. Investigation of many possible ways in which the disease may start indicates that the most common origin is thru the lodgment of the dry, wind-borne spores, either between the bases of two leaves where moisture remains after a shower or heavy dew, or on the surface of the bulb or neck during continued wet weather. The leaf tissue is specially subject to infection when the leaf is beginning to decline and turn yellow. Injuries to the growing plants, such as those made by insects and the breaking of the leaves by wind, or injuries to the bulbs during the process of harvesting or topping, afford ready access to the disease.

How the fungus destroys tissue.

The germinating spores send out thread-like tubes which pierce the skin of the moist bulb-scale and then divide and sub-divide both on and in the scale. The tips of the branches are provided with special organs of attachment which hold them to the surface of the scale until it can be penetrated and softened. Frequently these organs secrete a mucilaginous substance which aids in holding them in place until the tube can force its way into the onion tissue. Where the threads remain upon the surface or where they force their way there from within they unite in dense masses to form the characteristic bodies or crusts called sclerotia. These are covered with a dense black enamel-like coating. Thru this coating and also directly from the threads there grow long dirty-white or smoke-gray branches upon which are borne millions of spores to infect new points.

Factors affecting growth and fruiting.

Investigations to ascertain the factors which influence the growth of the fungus were made in the laboratory under very carefully controlled conditions upon pure cultures of it; that is, the fungus, and nothing else, was grown in dishes and tubes containing materials on which it thrived. Comparatively low temperatures proved most favorable to growth of the mycelium or fungus threads, the range within which branching and multiplication of these threads took place being from about 45° to 86° F., but very few spores were produced below 50° or above 80°. Excessive moisture induced rapid and copious growth of the threads and of the dark collections of these threads on the surface (sclerotia), but spore formation did not begin until the culture material was moderately dry. Free access of air was necessary for production of spores, but the threads and sclerotia were formed abundantly even in tubes of culture media with scanty air supply.

These factors explain the rapid development of the fungus in the cool, moist air of storage houses and the very profuse formation of spores when the rotting bulbs are exposed to warm, dry air.

The spores or conidia of the fungus, when again moistened, germinated freely after being held in a thoroly dry condition for two years, but their power of germination rapidly declined during the third year of such drying. The sclerotia, or crusts of fungus threads, when dry, would live for nine months to a year, but failed to grow after half that time if kept in moist earth. Both spores and detached bits of mycelium, then, may live in the soil long enough to infect the crop of the following year. The growing crop and the seed crop may thus be infected by the fungus on diseased tops or necks of refuse onions or directly by the fungus in the soil. Probably the dumping of decaying onions near fields on which a new crop is to be grown is the most dangerous source of infection.

Method of control.

Spraying experiments with bordeaux mixture, made in an attempt to control the neck-rot fungus, did not prove very successful, as there was only a slight decrease in the amount of onion shrinkage. There are certain difficulties in the way of successful spraying of onions, like the glaucousness of the foliage which prevents spray mixtures from adhering, and the breaking down and tangling of the leaves between the rows just at the time when spraying should be done to be effective. A combination spray of bordeaux mixture (5–5–50), Black Leaf 40 ($\frac{3}{4}$ pint to 100 gallons) and soap (5 pounds to 100 gallons) seems promising, as it adheres well and seems to control onion mildew and thrips, both of which injure the onion foliage so the neck-rot fungus gains easier entrance.

Fumigation of the onions with formaldehyde gas before placing them in storage was tested quite thoroly but proved ineffective, since the gas did not reach the living fungus threads which were already in the necks of the onions and closely crowded between the bulb scales. Furthermore, the gas failed to penetrate thruout crates of onions because of the usual amount of loose, dry skins. Also, the gas injured the bulbs in some cases. The average onion storage house is not tight enough to retain the gas.

Control of the disease must come thru careful cultural methods and providing proper storage conditions. Since most of the infection takes place late in the season, especially when the crop is ripening and curing, all possible means should be used to have the onions mature and the tops die early. Fertilizers that stimulate excessive growth, including rich manure, should not be used too freely, and not at all late in the season. Seed stock should be carefully selected to avoid types of onions with thick, succulent necks (" bottlenecks ") which are especially subject to attack. Air drainage should be carefully looked after since poor air circulation promotes onion mildew and, indirectly, increases the liability to neck-rot. The growing of onions in fields surrounded by hills or by dense woods should be avoided if possible; and tall weeds along ditches should be removed. The more quickly the bulbs can be dried the less the danger of infection with neck-rot. Since bruises make entrance of the fungus easy, great care should be used in harvesting and topping not to cause such bruises. Short tops — 1 to 2 inches — are probably better than longer ones.

After thoro drying in the windrows the onions should be screened to remove excess soil, tops and other refuse matter, thus making it easier to dry the crop in the crates. The bulbs should be kept in the cribs or drying sheds as long as possible, and placed in storage, wherever it can be done, in slatted crates of standard size, stacking the filled crates with 1 x 3 inch binders between to make ventilation easier. Putting onions in large, loose piles induces sweating that aggravates neck-rot.

Good circulation of air in the storage houses must be provided if neck-rot is to be controlled. Such houses should have ventilators at both top and bottom that can be opened and closed at will; and the temperature should be held as close to 35° F. as possible. Also, the house should be kept closed during warm spells in winter, especially if damp; otherwise the moist outside air rushes in, the moisture condenses on the cold bulbs and makes them so wet that neck-rot rapidly develops if the fungus is present. If heating is necessary, it is best to use stoves or dry heat, as steam pipes, when not in use become covered with moisture or with frost, which evaporates as soon as steam is turned on and makes the house damp.

Houses with low, flat roofs and poor top and bottom ventilation should be avoided, if possible; and all houses should be made double thruout, using a good quality of lining for the walls. Concrete houses should have air spaces between double walls and should be sheeted inside. Roofs should be of sharp pitch, with ventilators at the very top, and floors of heavy narrow pieces with half-inch spaces between them, especially in cellars or basements.

The old, rotting bulbs should not be placed on fields where onions are to grow, as they are a proven source of infection for the new crop, nor should they even be left near onion fields. Sound, healthy " mother bulbs " should be selected for seed fields, and it is best to treat seed to destroy fungus germs by soaking it 20 to 30 minutes in a solution of 1 ounce of formalin in 2 to 3 gallons of water. This may prevent onion smut, is a helpful precaution where onion blight is liable to occur, and may be valuable in preventing neck-rot, altho not many spores of the neck-rot fungus were found on the seed examined. The prevention of onion blight or onion mildew by spraying will have a marked effect upon the neck-rot which follows when poorly-cured, soft, blighted stock is placed in storage.

HOW BACTERIA IN MILK ARE COUNTED.*

F. H. HALL.

Until a few years ago no practicable method was known by which bacteria in milk could actually be counted. " Counts " have long been made, it is true, but these have been made by some cultural method in which the tiny plants themselves were not seen and counted, but " colonies " — little spots and circles that developed about the points where one or more bacteria were embedded in some jelly-like material suited to their growth.

Agar plate counts of bacteria.

Agar, a gelatinous substance derived from seaweed, has usually been the basis of this culture medium. Water, beef extract, peptone and often milk sugar are added to this, making a translucent jelly, fluid when warm, but solid when cold. A definite, small quantity of the milk sample is diluted with germ-free water to 10, 100, 1000, 10,000 or even more times its original volume and a measured amount of this dilution is placed in shallow, circular glass dishes. To this is added a much larger quantity of the warm and fluid agar; the mixture is then shaken to insure uniform distribution of the diluted milk and its contained bacteria. As the fluid agar hardens on cooling, the bacteria are caught and held wherever they happen to be. If the bacteria are single and separated in the diluted milk, then they lie well separated from each other in the hardened agar. If, as is more frequently the case, they are in groups of two to thousands of individuals, then each of these groups forms a single center of growth. These " agar plates " are placed where the temperature is right to induce the growth of the bacteria and then held for several days, usually either two days at a warm temperature, or five days at ordinary room temperatures. Bacteria multiply very rapidly, sometimes doubling in number every twenty minutes, so at the end of a few days, under these favorable conditions, each center of growth has become a mass of individual bacteria forming a " colony " large enough to be seen and counted. Unfortunately for the accuracy of this count, the tendency of the bacteria to grow in milk in masses which cannot be separated into their component individuals causes this count to be one of the number of centers of growth, rather than a count of the number of bacteria present. It has long been known that this was the case; but since there has been no available method of determining the amount of the error thus introduced, and since counts made from duplicate samples of milk agreed fairly well when made carefully, it has become the general custom to speak of these counts as counts of the " number of bacteria " in milk. So general has this custom become among American dairy bacteriologists, and so seldom is the fact mentioned that a single colony may represent *many bacteria* in the milk, not merely *one*, that " counts " of colonies and of numbers of bacteria have to most people meant the same thing.

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* Reprint of Popular Edition of Bulletin No. 439, November, 1917; for Bulletin see p. 200.

Counts on new basis.

In 1911, however, a modification of earlier and little used microscopic methods of counting bacteria in milk was announced, which has since been perfected, and which possesses many advantages over the plate method for the counting of bacteria in unpasteurized milks, so that it is being rapidly introduced in milk inspection work. Instead of counting " colonies " after their development on a culture medium, the actual bacteria are counted under a high power microscope in the original milk distributed in a thin layer or " smear " on a glass slide and dried. By the microscopic method the results can be secured within a half hour of the time of sampling the milk, and the microscopic slides may be preserved as a record of the condition of the milk. The milk smear is cleared of fat. The bacteria are stained so that they show plainly and are killed in preparing the slide; therefore, no growth or colony development takes place.

By this method, also, the form, shape and size of the bacteria can be studied, giving much information regarding their character; and the actual numbers of the germs can be counted, whether they be alone, in pairs, in chains, or in groups. Occasionally groups are found so large and compact that the numbers must be estimated, not counted, but such groups are uncommon.

New method gives high counts.

Unless some uncontrolled error creeps into one or the other counts, the counts of individual bacteria by the microscopic method greatly exceed the counts of colonies obtained by the agar plate method. Consequently, consumers and others who take an intelligent interest in the question of milk quality must be warned that as the new method comes into more common use, they may expect larger numbers of bacteria to be reported as found in milk. The actual numbers of bacteria in the milk will not be larger, but the microscopic method will give figures that represent more closely than does the plate method the number of germs really present.

Errors in count.

Because of the physical impossibility of counting the hundreds of thousands or even millions of bacteria which may be present in a single drop of milk, all counts are made from a very small amount of material. The final figures given, obtained as they are by multiplication of the count made, by a figure of the proper size to give results per cubic centimeter of milk, are in every case to be regarded as " estimates " rather than as " exact " counts. As estimates they are subject not only to the usual errors of estimates, but they are also subject to certain other errors which can be made plainer by a comparison with counts of more familiar objects, such as seeds.

Counting seeds compared with counting bacteria.

Suppose a farmer had several quarts of accidentally mixed seeds in which there were beet, clover, timothy, lettuce and tomato seeds, each quart unlike every other in number and proportion of the different kinds of seeds; and he wished exact information regarding each quart. After thoroly mixing them, he sows one one-thousandth of the seeds in each quart over circles containing one square rod each of clean soil, distributing them as evenly as he can. Now he can wait a few weeks to make his counts until the seeds have grown into seedling plants. He then selects a few areas each containing one square foot and counts all of the plants which have germinated from the seeds. Or, he can make his counts at once by distributing the seeds in a like fashion on white cloth, marking off areas of one square foot each upon the cloth and counting the seeds as they lie sharply contrasted against the background.

Certain errors may occur in the first count very similar to those which occur in making agar plate counts of bacteria. To make the comparison exact, we must assume that he is unable to distinguish the separate plants produced from each beet seed ball, so that all groups of beet plants are counted as if they were single plants. It may be assumed also that part of the tomato seed falls on ground that is so wet that it does not grow, altho it would have done so under proper conditions; or that some of the clover seeds fall on acid places in the soil and do not germinate. Some of the seed may also have been dead when they were planted. Assuming for the moment that he wishes a count of viable seeds only, then the latter may be disregarded. The other errors will all tend to make the count too low. But, however careful he may have been in preparing the soil for his seed count, seeds of weeds or hold-over seeds of these same kinds are liable to be present, to germinate and be counted; so his figures may be much too high. He cannot make any count of the seeds which will include the dead seeds as well as the living ones.

If, however, he should count the seeds at once, as collected on the square foot areas of white cloth, he can see how many true seeds there are in each seed ball of the beet, and can count each of the other seeds separately. If he does not have a magnifying glass, or his eyes are poor he may overlook some of the small, light-colored timothy seeds, causing underestimates of the number present. On the contrary, all of the seeds present, including dead as well as viable seeds, will be seen and counted. In some cases this may be the count desired; but in others the desired count may be one of viable seeds only. In the latter case supplementary germination tests would show whether the error caused by the presence of dead seeds was significant.

The comparison between the difficulties met with in making satisfactory counts of these mixed seeds in the two ways just described are so nearly identical with those met with in making counts of bacteria by the agar plate and the microscopic methods that it will require very little explanation to make these difficulties clear. Who can doubt, however, were the two methods mentioned above the only ones by which a farmer could compare and count his seeds, that he would use the one in which he could see and count the actual seeds rather than the one by which he had to wait a considerable time and estimate the number of seeds from the plants produced? And this would be especially true if the error introduced by the compound nature of the beet seed balls was a large one. By the time the seeds have germinated and the plants appeared it might be altogether too late to use the knowledge in selecting seeds for the season's planting. Moreover, if the counts were to be used as standards for judging the quality of the seed from the commercial or the control standpoint, many disputes over the counts could be prevented and fraudulent practices stopped by the preservation of the samples of seeds; while the germinated seeds could not be preserved.

Application to counting of bacteria.

During the past few years some work done by the Bacteriological Department of the Station has given a good opportunity to compare the "counts" made by the two methods of counting bacteria, and to analyze them in such a way as to determine which of the errors discussed above are really important in making routine counts of bacteria in milk. The samples, 643 in number, were taken in the morning from cans brought to the City of Geneva for its milk supply, and were about equally distributed between night milk, usually about 16 hours old, and morning milk, not more than four hours old. The samples were brought directly to the laboratory, and plates for the agar counts and slides for the microscopic count were prepared as quickly as possible under conditions which made the resulting comparisons fair ones. The plates were held for five days at room temperature and counted; then placed for two days at a higher temperature and counted again. Usually the latter count was the higher, owing to the development of new colonies from bacteria which do not grow at the lower temperature; and the higher count was used. Only " colonies " could be counted on the plates; and there was no way of telling whether these had developed from single bacteria or from many. Under the microscope the bacteria could be plainly seen, and two kinds of counts were made; one, of the whole number of individual bacteria, and the other, of the groups of bacteria. In the " group " count each individual separated from all others was counted as a " group," as well as the real groups of two or more bacteria.

If no irregularities occurred in the counts due to the presence of dead bacteria, or of organisms that failed to grow under these conditions, or to extra colonies on the plates developed from contaminations, or like things, then the counts of " colonies " on the plates should be greater than the number of " groups " and less than the number of individual bacteria. This proved to be the case in more than half of all of the counts (54 per ct., or 345 out of the 643 samples). If, however, all of the counts in which the number of individual bacteria was less than 30,000 per cubic centimeter are excluded (200 in number), nearly three-fourths of the remaining counts (71 per ct. of the 443 samples) show this normal relationship. These figures indicate then that the chief reason why agar plate counts tend to be smaller than the microscopic counts is because the bacteria exist in the milk as groups more frequently than as single individuals; and that irregularities in counts due to other causes are less important than those due to the clumping of bacteria.

Because of the importance of this effect, a further study was made of the average size of the " groups " of bacteria found in the milk, this being easily determined from an examination of the microscopic preparations. Thus, 3600 groups selected at random from milk of all grades were found to contain an average of 11.8 individuals; but these groups varied greatly in average size according to the type of bacterium. Thus " groups " of bacteria of the ordinary lactic acid types were found to be of small size, containing an average of only 3.1 individuals; while those of the streptococci (organisms which occur commonly in milk, frequently being derived from cows with garget) showed " groups " containing an average of 33.7 individuals. The average size of the " groups " in 580 of the 643 samples was 7.9; but there seemed to be a tendency for the size of the " groups " to vary with the grade of the milk. The better grades of milk containing very few bacteria showed " groups " containing an average of 2.2 individuals. The " groups " increased in size from this to an average of 12.9 individuals, and then again decreased to an average of 5.6 individuals as the bacteria developed in number and there came to be more than 10,000,000 of these organisms for every cubic centimeter (about one-third teaspoonful).

Effect of grouping of bacteria on count.

In the making of the agar plates the samples of milk are diluted with germ-free water from 10 times to 10,000,000 times or more and these groups of bacteria are more or less broken apart; but they are never so broken apart that the resulting colonies each represent a single bacterium. In the making of the " smear " on the microscopic slide on the other hand, only a small portion of milk is taken, but this is not diluted, so the bacteria remain in groups as they were in the original sample. It has thus far proved impossible to control or measure the effect of the breaking of the groups in such a way that it is possible to correctly estimate from the plates what the total number of individual bacteria would be as seen under the microscope; nor to reverse the process and tell from a microscopic slide what the probable agar plate count would be in the case of individual samples of milk.

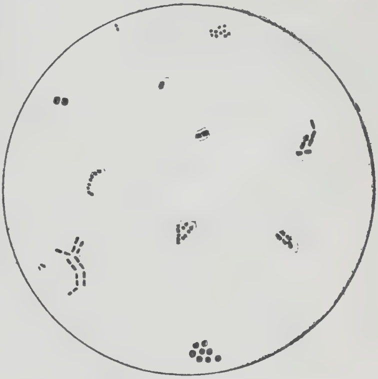
Effect of dead bacteria on count.

A few cases occurred (19 per ct., or 123 samples out of 643) in which the colony count did not even equal the " group " count, to say nothing of the individual count. Why is this? It must be remembered that to form colonies on the plates the bacteria must grow. To grow they must be alive, and even if alive must find conditions suitable for growth, just as in the case of the seeds. Bacteria are plants, and die as do other living things. The question, therefore, arises whether any of the discrepancies between the agar plate and the microscopic counts may be due to the presence of these dead bacteria. If such were present in the samples examined they must be included in the comparatively small group of samples where the plate count was smaller than expected. That the presence of dead bacteria was not the cause of all or even a majority of the 123 counts of this type, is indicated by two things; first, that bacteria almost invariably thrive and grow rapidly in fresh milk, and second, that the same effect is produced by the presence of living bacteria which do not find conditions suitable for growth on the agar even where conditions are favorable to their growth. Every gardener knows how difficult it is to get every seed to grow. Just in the same way, bacteriologists have found it impossible to so prepare a culture medium as to make it suitable for the growth of all bacteria. So well known and common is this effect that it is reasonable to assume that this played the larger part in causing the 123 samples to show the low plate counts.

Effect of contaminations in plating.

In discussing the counts of seeds it was pointed out that hold-over seeds might grow and thus increase the count. In the series of 643 counts there were 175 samples (27 per ct.) in which the plate counts were larger even than the individual counts as made under the microscope. In a very few instances the discrepancy was very large. Yet a careful renewed search thru the microscopic preparations failed to show any such large numbers of bacteria as were indicated by the agar plates. The most natural explanation of these irregularities is that extra colonies developed on the plates from bacteria which got into them from outside sources, and this was taken to be the case until it was noticed that all but 32 of the 175 counts were made on milk samples containing less than 30,000 individual bacteria per cubic centimeter. The localization of the majority of these counts among the milk samples containing few bacteria indicates that the majority of the counts of this type were caused thru the mathematical impossibility of getting a fair average when only a small number of objects are counted, from which to estimate a very much larger number.

In making a microscopic count, it must be remembered that we do not examine the whole of the "smear" on the slide; since the field viewed under the microscope is exceedingly small and it would take altogether too much time to pass all of the thousands of fields in each smear under the microscope. Accordingly, we examine only one hundred of these fields, count the number of bacteria found, and multiply this number by the proper factor to give the approximate number per cubic centimeter. If the milk contains only a very few bacteria, we may not even find one in one hundred fields of the microscope, and so use too small a number in the computation. On the plates a much larger fraction of a cubic centimeter of milk is examined in those milks which contain few bacteria than in the case of the microscopic slides. In making comparisons of counts from low-count milk samples, the probability is that the microscopic count will be lower than it should be. This is quite probably what happened in the 143 cases in which the colony count on the agar was greater than the individual count on the slides. Many of these discrepancies were not large, and under these conditions where so few bacteria were present the microscope makes no mistake in placing it in the "good" or Grade A class even tho the number of bacteria found is not quite as large as the number found by the plating method. No difficulty of this sort arises in milks containing a sufficiently large number of bacteria to be found readily under the microscope. In the case of high-count milk, the



Groups of Bacteria in Poor Quality Milk as They Appear Under a Microscope.

advantage of examining a relatively large amount of milk is held by the microscopic and not by the plate method. It is possible that some bacteria are so small, or stain so lightly, that they are commonly overlooked under the microscope altho they would form colonies on the agar plates. There are few indications, however, of such instances, and it is believed that they are rare. The effect of such varieties if present would be to make the microscopic counts lower than they should be.

Comparison with counts of seeds. Let us return for a moment to the hypothetical methods of counting seeds. The results of the studies on the counts of bacteria show that the commonly used agar plate counts are much too low because of an error similar to the one caused by counting the beet seed balls as a single seed. They also show that there is an error of smaller size due to the presence of living bacteria which do not grow; an error similar to the one caused by the failure of some of the seeds to grow. The indications are also that where the work of making bacteria counts is carefully done, errors due to contaminations, while they do occur, are much less important than would be the similar error caused by weed seeds present in soil.

None of these errors affect the microscopic counts, the errors in this case being largely those which come from the difficulty involved in making accurate counts of objects so tiny that they can not be seen except with high magnification under the microscope. The claim which is frequently urged against making counts of bacteria microscopically based on the fact that dead bacteria cannot be readily distinguished from living ones does not prove to be of any significance in the case of fresh unpasteurized milk; because of an apparent absence or practical absence of dead bacteria in such milk. There are circumstances, moreover, in which a total count of the bacteria is of greater importance than a count of the living bacteria; and in fresh milks the dead bacteria give just as much information concerning the past history of the milk as do the living ones. Under certain special circumstances where preservatives have fraudulently been added to the milk, it is especially desirable to be able to detect the dead bacteria; a thing which cannot be done by means of the agar plating method.

Conclusions. In view of the manifest impossibility of so perfecting either the agar plate method or the microscopic method that absolutely accurate counts of bacteria can be made; or even of controlling the errors which have been discussed, it is fortunate that comparisons of real value can be made without counts more accurate than they now are when properly made. There is no necessity for us to be able to count the bacteria in milk so accurately as to tell the difference between a sample containing 5,657 bacteria and one containing 6,756 bacteria. In looking at a distant hillside, it is not possible to count the trees thereon. Nevertheless, it is ordinarily quite possible to form an accurate judgment as to the area covered by woods, by wooded pasture, and by meadows. Such a judgment may have as great a value for the purpose in hand as would one based upon an accurate count of the number of trees per acre of land. The fact that accurate counts of the number of bacteria in milk cannot be made does not prevent the use of either the agar plate method or the microscopic method as a means of distinguishing with a satisfactory degree of accuracy between milks containing few bacteria, a medium number, and a large number. The fact that this is all that is necessary for commercial and control work has been shown in the inspection work which has been done for the City of Geneva during the past three years, a description of which will be given in a forthcoming bulletin.

It is evident from all of this work that popular ideas of the number of bacteria in milk, based as they are upon the counts made by the plating method, must be revised. The results obtained by the microscopic method of counting bacteria in milk show these to be much too low.

CONTROLLING A RADISH PEST.*

F. H. HALL.

A triple-action pest.

A serious pest with which the grower of truck crops must contend is the cabbage maggot, or radish maggot. This little grub, the larva of a fly much like our common household nuisance, often ruins the prospect for a good crop of early cabbages; it so tunnels, rasps and weakens the roots of plants of late cabbage in the seed-beds that the grower must frequently send away for his plants, and it all too commonly mutilates and deforms mid-season radishes so that only a small portion of the crop is fit for the table.

The work of the insect as a cabbage pest has been discussed in a previous bulletin of the Station (No. 419); but its activities on the radish, tho of less economic importance, are undoubtedly better known to the home gardener. Hardly a season passes that the tunnels of the little white grubs are not found so abundant in many radishes that these must be thrown away. Occasionally the numbers of the pest have been so great that it has been practically impossible to get a perfect radish in early or mid-June.

Time of injury.

The radish is a spring and summer crop, and the maggot flies appear at just the right time, from the second to the last week in May, so that their eggs are laid and the little grubs hatch and become active at their destructive work just when radishes in most home and farm gardens are making their best growth. Radishes that reach pulling size before or during the first week in June, in normal seasons, usually escape injury from the maggots; but to have the roots ready by this time the seed must be sown in March or early April. This is much earlier than most gardens are ready for planting. Such early sowing of radish seed is necessary to have the roots mature in late May or early June, since the radish does not grow very fast until the soil warms up somewhat. In tests extending over four years, it was found that it required two months or more to grow radishes from seed sown during the last half of March, about seven weeks to grow them from seed sown in early April, six weeks from seed sown in late April and only four or five weeks from seed sown in May. The maggots disappear soon after the middle of June, but radishes maturing after this time, except under very favorable conditions, are liable to be tough and too pungent for satisfactory use. To grow good radishes without protection, then, it is necessary to prepare the ground and sow the seed just as early as weather conditions and the physical condition of the soil will admit.

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* Reprint of Popular Edition of Bulletin No. 442, November, 1917; for Bulletin see p. 460.

[Plates XXXI and XXXIII illustrated this text.]

Protection possible.

But investigations made at the Station prove that the remedy that works so well for the grower of cabbage plants for the late crop is also valuable for the radish grower. This is, to cover the beds with cheesecloth.

During all four years of the tests, 1914–1917, both the radish maggot and flea beetles were well controlled by the use of such cheesecloth screens; and in three of the years the yields of radishes were much increased by this method. In 1917, owing to the wet, cold spring, the number of maggots was much reduced. Injuries to radishes, whether screened or unscreened, were comparatively slight, and the increase in yields under screening was much less than in previous years. The summarized data for the four years are shown below:

Table I.— Summarized Yields of Screened and Unscreened Radish Plats from 1914 to 1917.

(Counts and weighings are based on rows of one hundred feet in length.)

	Plants.	Weight of plants.		Marketable roots.	Weight of marketable roots.		Average weight of radish.
	No.	*Lbs.*	*ozs.*	*No.*	*Lbs.*	*ozs.*	*Oz.*
Averages for screened plats, 1914	456	8	8	236	3	6	.23
*1915	519	5	13	213	2	13	.21
1916	742	9	0	487	5	5	.17
1917	1,089	16	6	596	6	8	.17
Averages for unscreened plats, 1914	370	3	9	76	0	10	.13
*1915	624	3	8	110	0	13	.12
1916	650	4	3	211	1	15	.15
1917	1,063	13	2	568	5	13	.16
Total average of screened plats per 100 feet	702	9	12	386	4	8	.19
Total average of unscreened plats per 100 feet	677	6	2	241	2	5	.14

* One plat only.

Tests and results. This summary results from extensive tests, including from five to ten plats during three of the four years, with seedings at week intervals from March 15 to May 18. The plats were 100 feet long and one or more rows were sown the entire length of each plat for each seeding. The screened beds were carefully covered with the cheesecloth (from 20 to 30 meshes to the inch) and the bottoms of the side and end boards were carefully banked up with earth to keep out the maggot flies and flea-beetles.

The differences between the screened and unscreened beds have usually been apparent almost from the time of sowing the seed, as the slight cover given by the thin cloth retains moisture and increases the temperature of the screened bed. Without such protection the surface dries out and, on heavy soil particularly, forms a crust that interferes with the little plants. As a rule the seedlings in the open beds were one or two days behind the screened plants in the time of their appearance above the ground, and the radishes under the cloth grew more rapidly and seemed more vigorous thruout the growing season. The roots grown under protection were usually more succulent and tender and more satisfactory for market, while the leaves, also, were larger, smoother and less leathery.

Screening has usually kept out practically all of the maggot flies and many, though not all, of the flea-beetles. The work of either insect has been negligible on the screened beds; but on the open beds both have worked destructively.

The flea-beetles were usually first to appear, and ate small round holes through the cotyledons of the tiny radish plants. Young seedlings frequently died from the first attacks of these insects; and others were so stunted by their continued work that they never produced roots of marketable size.

Very soon after the maggot flies emerge from the ground, which may be during quite a period extending from early May to mid-June, the females lay their eggs in some crevice or crack on the stem of the radish plant at or a little below the surface of the ground. The eggs hatch in four or five days and the larvæ — maggots — attack the roots. They make tunnels in the tissue or grooves on the surface which injure the radish or deform it. Their work may often be followed by decay of the injured tissue.

Other pests under screens. During the past two seasons, two other pests have worked on radishes; and have apparently been worse under the screens. These are "slugs," or shell-less snails, of three species, and millipedes or "thousand legs" of two species. These are not insects, but their work is similar in many ways to that of radish maggots. The snails rasp and eat the leaves, and both snails and millipedes eat holes in the radishes so that the white tissue shows through the red skin over large or small areas.

Both of the creatures prefer cool, moist weather, and found the spring and early summer of 1916 and 1917 much to their liking, so that both, but particularly the snails, were more abundant and more harmful than ever before. As the screened beds retain moisture better than open beds and the large radish leaves developed there shade the ground and make it cool, both pests seemed more abundant on such beds than in the open.

The snails were destructive to many other plants, especially in gardens, such as lima beans, corn, dahlia, potato, chicory, dandelion and lettuce. They also mutilate ripening strawberries. They work mainly at night when they may be found by using a lantern or flash light and be destroyed. They may also be trapped by using slices of raw potato, which they seem to prefer to almost any other food. Attempts to poison them have not been successful as they seem very resistant to arsenicals altho they eat the poisoned baits freely. They seem to find the copper sulphate of bordeaux mixture distasteful, however, and will avoid plants coated with this spray.

The remedies that may be used with fair promise of success are applying dry air-slaked lime about the plants, collecting the snails at night by lantern light, trapping them with sliced cull potatoes, and spraying with bordeaux mixture as a deterrent. The millipedes do comparatively little harm, fortunately, since no methods of control for them are known.

USING THE MICROSCOPE IN MILK GRADING.*

F. H. HALL.

A rapid, reliable method.

The number of bacteria in milk has been used for more than a decade by certain milk distributing companies as a means of judging the commercial quality of the milk they received. The abundance of bacteria does not alone determine the quality of milk, by any means, but does furnish a guide by which we may judge of the keeping quality of the milk and make an approximate estimate of the time limit of its usefulness in fresh condition. Until recently this application of the bacterial count to the milk industry has been made by a method which could not give the counts until after the milk had been used or had soured; that is, the agar plate method reported conditions which were already *several days* past. It was useful in showing what kind of milk, from the bacterial standpoint, a producer had brought to market some time before; but it could not give notice, in time to be of much use, of any transitory change in the quality of the milk of a herd or any particular cow in it. By this method the bacteria in milk diluted with germ-free water were allowed to grow for from two to five days in a shallow glass dish containing a " culture medium," when the separate bacteria, or collected groups of them, developed " colonies " or masses large enough to be seen and counted. By the microscopic method, whose practical application we are to discuss, a small portion of the milk is spread on a small rectangular piece of glass and dried, then prepared by immersion in liquids and staining solutions in such a way as to make the bacteria visible and the whole suitable for examination under the high magnification of a compound microscope. This slide, which forms a permanent record of the bacterial condition of the milk, can be prepared in ten to fifteen minutes and the preparation examined at once, or it can be kept until some convenient time. The rapidity and simplicity of the method are sufficient to permit examination of the bacterial quality to be made before the milk is consumed or placed on sale. By this method the individual bacteria are seen and counted, whether each is separated from all others or many are united in a clump. These counts are therefore greater than those obtained by the ordinary methods in which clumps of bacteria are counted as if they were single bacteria. All of these facts give the microscopic method many advantages over the generally used plating method for judging the quality of milk produced on farms.

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* Reprint of Popular Edition of Bulletin No. 443, December, 1917; for Bulletin see p. 244.

Practical tests made.

Preliminary tests of this method made in 1913 gave very promising results and it was decided to try it out under commercial conditions and in comparison with the plating method. This test was made at Hobart, N. Y., in 1914 and proved the new method at least as valuable and as reliable, if not more so, than the older one; so it was used alone for more than two years to grade the milk of the City of Geneva. For two full years of the time it was accepted by both dairymen and milk distributors as a guide to the payment of premiums for good quality milk.

Tests at Hobart.

The preliminary tests were made in a laboratory operated by the Lederle Laboratories for one of the largest milk distributing companies of New York City. This Laboratory tests the milk received at Hobart for butter making, and that brought to three pasteurizing plants at Bloomville, South Cortright and Cobleskill for shipment to the city. At the shipping stations premiums are paid for milk with low bacteria counts, but at Hobart no such stimulus to the production of milk with a good bacterial quality was offered, and only 50 per ct. of the milk brought in there graded A, while the supply at the shipping stations averaged 80 per ct. Grade A.

The grading of the milk was based on the agar-plate counts made by an assistant of the Lederle Laboratories, and the microscopic slides were made at the same time, from the same milk samples, by an assistant bacteriologist of the Station. The gradings were made independently.

During February, March and April, 1504 samples of milk from the three Grade A pasteurizing stations were examined by both methods. The ratings made by the microscopic method were found to agree with those made by the plate method on 1339 samples, over 89 per ct. When the two methods disagreed the microscopic method gave too low counts in a little more than half the cases, and the plate count gave too low counts in the others. In the following July, 426 additional samples of milk from the same stations were graded by both methods, with an agreement in 85 per ct. of the comparisons. Many of the discrepancies in both winter and summer work were due to slight variations above or below the number fixed as the limit for Grade A milk; so that most of the error in grading really falls within limits that would be considered reasonable in any experimental work; but there were occasional wide variations in both directions.

These variations, small or great, so offset each other that the totals of samples placed in the two grades by either method agreed very closely. Thus out of 1504 samples examined in cold weather, 1280 were graded A by the microscopic method and 1270 by the agar plate method; and of the summer samples 212 were graded A by the microscopic method and 234 by the plate method. This is a surprisingly close agreement and is probably as good as could have been secured if duplicate analyses of the same samples had been made by the agar plate method.

Microscopic method used at Geneva

So satisfactory was the agreement between the two methods as used at Hobart, it was thought there could be no unfairness to anyone in using the microscopic method alone in grading the milk received at two milk stations in Geneva, especially as slight changes in the details of the method promised even better results than were secured in the comparison tests. After a preliminary period of two months, the two milk distributing companies of Geneva and the dairymen who sold the milk agreed to accept the grades as determined by the new method. These grades were made to conform as nearly as possible to the three grades established by the State Sanitary Code designated by the letters A, B and C. To prevent confusion with these, the ratings given by the microscopic examination were called "good," "medium" and "poor." Because of the fact that the individual bacteria are seen under the microscope, the numbers used as the limits between the grades given microscopically are necessarily much higher than those used in the Sanitary Code for milk of similar quality. Thus the milk with less than 1,000,000 bacteria per c. c. was graded as "good," that with 1,000,000 to 10,000,000 "medium" and that with more than 10,000,000 as "poor." Actual counts were not made unless the sample appeared to be close to the limits between the grades. Usually an examination of 30 "fields" of the microscope was sufficient to place the milk in its proper grade, without counting.

The samples were collected at least once weekly from each can of milk delivered at the milk stations, temperatures taken, and records kept to show which samples were taken from night milk and which morning milk. As a check upon the accuracy of such weekly sampling, each can of milk brought in was tested every day during two periods of one week each. Reports showing the grading of each dairyman's milk were sent after each examination, and if anything of special interest developed in the milk of any patron at any time, he was informed of it by telephone. Many of the dairymen visited the Station laboratory in response to such messages, often coming when it was possible to show them the entire process of making the microscopic preparations, and to have them study the milk thru the microscope. The permanence of the slides also made it possible to show any skeptic why his milk had been given a low grade. The rapidity and simplicity of the method, the visibility of the tiny germs themselves under the microscope, and the permanence of the preparations make such grading of the milk easier to understand than that by the older, indirect, and slower agar plate method.

As payments for the milk were based on the monthly grades, and as some cans from a producer would at times vary in quality from the majority of them, 25 per ct. of poorer milk was allowed in each grade without changing the rating; but in computing the 25 per ct. allowance each can of " poor " milk was considered equivalent to three cans of " medium " milk. Thus a producer who marketed during the month, 16 cans of " good " milk, 3 cans of " medium " milk and one can of " poor " milk would receive a " medium " rating. In this case the percentage was computed as if he had delivered 22 cans of milk, 16 of which were " good " and six " medium " in quality.

In this work at Geneva, nearly 12,000 cans of milk were examined, of which about 86 per ct, were found to contain less than 1,000,000 bacteria per c. c. and were rated as " good," 13 per ct. were graded " medium " and a little over one per ct. contained more than 10,000,000 bacteria per c. c. and so were rated " poor." Lest anyone should be surprised that milk containing as many bacteria as 1,000,000 per c. c. should be rated as " good," attention is again called to the fact that these are counts of the bacteria themselves, and not counts of groups of bacteria such as are obtained in the officially recognized agar plate count. The " good " milk was all of such a grade that it would have satisfied the standards ordinarily set for milk to be pasteurized and sold under the Grade A label, and therefore really deserved this rating.

A comparison between these findings and a series of analyses made from 20,000 samples taken by the New York City Department of Health in 1912 at 432 milk stations supplying milk for the city, indicates that the quality of the milk received at Geneva was as good as, if not better than, the milk received at that time for New York. The Geneva milk, on the other hand, did not average quite as good in quality as that received at the three Grade A pasteurizing stations and tested at Hobart; but was better in bacterial quality than that brought to Hobart for making butter.

Some high-count milk was found at Geneva in winter as well as in summer, and some low-count milk in summer as well as in winter. The smallest percentages of " medium " ratings were found in May, 1915, and March, 1917; but the percentages for August, 1916, and

January, 1917, were not much higher. The milk as a whole showed the poorest condition in July, 1916, and September, 1915.

Treatment of milk cans very important.

Two companies handle practically all of the milk sold in Geneva, and the examinations showed marked differences in the bacterial counts of the milk received at the two companies. Not all of the factors responsible for this difference could be located, but some of them were, quite definitely, and correction of the poor conditions brought immediate improvement in the milk.

GROUPS OF BACTERIA IN POOR QUALITY MILK AS THEY APPEAR UNDER A MICROSCOPE.

Insufficient steaming of the milk cans over a steam jet, with improper care given the cans after steaming, was a leading cause of high counts in the milk; or at least a marked decrease in the counts followed simple changes in the manner of handling the cans. At first, the cans at one receiving station were washed, rinsed with a jet of water, steamed for five to ten seconds, covered and allowed to stand upright, in which position the steam condensed leaving the moisture in the cans. As shown by investigations made elsewhere, cans handled in this way, when left standing for 24 hours or longer at temperatures favoring bacterial growth, may develop millions and frequently billions of bacteria. By inverting the cans and leaving the covers off, conditions were improved considerably altho the cans did not dry perfectly. Later, some cases of "medium" and "poor" milk were investigated, and these drained cans were found still to contain millions of bacteria that could be washed out with sterile water. Another change in the manner of handling the cans was made, by which the cans were steamed for twenty to thirty seconds, instead of five or ten, making them so hot that they dried out quickly after draining out any condensed steam. They were then found to be in good condition bacteriologically, and the amount of "poor" and "medium" milk brought in by the producers again decreased. However, other changes in conditions made at the same time may have aided in this reduction of bacteria.

Milking machines need care.

Several of the herd-owners selling milk to this company used milking machines at some time during the two years. Most of these herds were large and the operation and care of the machines were left to hired help who did not appreciate the necessity of following directions in regard to the care of the machines.

These directions involve the use of mild germicides with whose action on bacteria they were unfamiliar. As experience at the Station has shown conclusively, milk with low bacteria counts can be drawn by machines under practical conditions by using simple and inexpensive precautions, but inattention to these precautions is fatal to success.

On one farm, where the owner of the herd did most of the work himself, the introduction of a milking machine caused no decrease in the grade of the milk, and bacteriological examination of the machines indicated that they were kept in good condition, sterile water milked thru the machines showing very few bacteria. On the other hand, milking water thru a machine used on another farm showed millions of bacteria in the water first passed thru, and almost as great a number in the second " milking " of sterile water.

On all of the six farms where machines were used except that operated by the owner, and possibly one other, the bacterial quality of the milk was lowered after machine milking was introduced, or was improved after the use of the machines was abandoned. The bacterial quality of the milk from the six farms was about the same as that of an equal quantity of milk produced on the eleven farms which supplied the poorest quality of hand-drawn milk.

These facts must not be misinterpreted. They indicate that the machines were not given proper care, not that they themselves were at fault.

However, the improper care of the machines, all but one of which (the one given the best care) happened to be owned on farms sending milk to one of the two companies, is one very good reason why the milk received by that company did not grade as high as that of the other company.

Detection of garget.

One of the advantages of the microscopic method is that it makes possible prompt detection of " gargety " milk, none of the other tests which have been used in sanitary milk control work giving results which are as satisfactory for this purpose. The prompt reports given on this type of milk enabled the owner to withhold the product of an affected cow and so maintain his grade. Accompanying gargety conditions of the udder is a type of bacterium which grows in long chains like a string of tiny beads that do not readily separate, and can easily be detected in microscopic preparations. The presence of this germ in the milk, when abundant, would be reported to the owner at once, and, if necessary, samples of the milk of the different cows in the herd be taken to locate the guilty one. In nine instances these individual samples were taken and the offending animals located at once; and in several other cases the owner was asked to look for " gargety " cows and was able to locate them without further bacteriological examinations.

No connection could be found between milking machines and garget in the herds, extensive infections with garget appearing at times in both hand-milked and machine-milked herds. All but 8 of the 36 men whose milk was tested continuously thruout the period of 26 months brought in, at some time, milk showing large numbers of this streptococcus or chain-forming bacterium, such milk comprising more than one-fifth of all that graded "medium" or "poor."

Comparison of methods.

During the progress of this work, 643 samples of milk were examined by the agar-plate method as well as microscopically, and much better agreement between the results of the two methods was obtained than at Hobart. It is somewhat difficult to adjust the relation between the A, B and C grades established on the plate count basis, and the good, medium and poor milk as determined by the higher counts shown under the microscope; but making the comparisons as closely as possible, the two methods gave results which agreed in more than 91 per ct. of the cases. This, compared with the 85-89 per ct. of agreement obtained at Hobart, where but two grades were used, is a very great improvement. The discrepancies were again equally due to too high and too low readings by either method.

Daily or weekly testing.

In order to learn, if possible, whether one test a week of each producer's milk gave a just basis for grading, a sample was taken from each can of milk delivered to one of the companies during one week in August, 1915, and one week in February, 1916. This extensive testing involved taking and examining from 30 to 50 samples daily; but the work was all easily done by one man and the results for each day were known before or shortly after noon.

Decided differences were shown in the bacterial quality of different cans of milk from the same herd, even on the same day; so that the records secured were not regarded as extensive enough or detailed enough to decide definitely how frequent sampling should be to give reasonably accurate grades. It is important that further studies be made along this line if milk is to be bought and sold extensively according to its bacterial count.

In the case of 17 men whose milk was tested during these two seven-day periods, frequent sampling gave no different grading from that of the month, based on weekly testing. The work did prove, however, that both morning and night milk should be tested. As the night milk, delivered in the morning, is 12 hours older than the morning milk, the bacteria in it have had time to increase and the added number may be enough to lower the grade of the can. In the winter week only three cans of "medium" milk were found in the morning milk against ten cans in the night milk. In the August week, when summer temperatures as well as the longer time of holding promoted development of bacteria in the night milk, there were four times as many cans of lower grade milk in night milk as in morning milk. Evidently if the samples had been taken only from the night milk, injustice would have been done the producer; if from the morning milk alone, to the purchaser.

Conclusions. The work at Hobart proved that the agar plate and microscopic methods agreed closely in results when used simply to divide milk into two grades, and the variations between the two methods were about equally balanced in direction. This agreement is probably about as close as would have been obtained in making duplicate tests of the same number of samples by the older method. As there is no means at present available by which we can know the exact number of bacteria in a given quantity of milk, it seems fair to assume that use of the microscope in grading gives results which are at least as accurate as those secured by the agar plate method.

The more carefully controlled experiment at Geneva, continued for twenty-six months, shows that it is entirely feasible and practicable to use the microscopic method for dividing milk into the three grades recognized in the Sanitary Code in use in the State. The labor involved and the expense of equipment are much less than that for the plate method, the results are available within a few minutes or hours as desired, and the slides can be preserved indefinitely. This permits the checking of fraudulent practices in grading in a very satisfactory and simple way and leaves a wealth of material for detailed study at any time.

Microscopic examination of the milk was found to be very helpful in detecting some causes of excessively high germ counts, especially in locating cows with garget. Other causes were not so easily identified. Two other factors were held to be responsible for high counts in the Geneva milk: (1) Improperly steamed and poorly drained cans that stood with their covers on where warmth promoted the growth of bacteria; and (2) milking machines that were not kept free from bacterial growth.

The whole series of studies proves the microscopic method worthy of consideration by all who in any way use the bacterial content of milk as a guide in commercial grading.

PERIODICALS RECEIVED BY THE STATION.

Abstracts of Bacteriology	Subscription
Agricultural Digest	Complimentary
Agricultural Gazette of Canada	Complimentary
Agricultural Gazette of New South Wales	Complimentary
Agricultural Journal of India	Complimentary
Agricultural Review	Subscription
American Agriculturist	Subscription
American Chemical Society Journal	Subscription
American Entomological Society (Transactions)	Subscription
American Fertilizer	Subscription
American Florist	Subscription
American Food Journal	Complimentary
American Fruit Grower	Complimentary
American Grocer	Complimentary
American Hay, Flour and Feed Journal	Complimentary
American Journal of Botany	Subscription
American Journal of Physiology	Subscription
American Miller	Complimentary
American Museum of Natural History, Bulletin	Complimentary
American Naturalist	Subscription
American Philosophical Society, Proceedings	Complimentary
American Poultry Advocate	Complimentary
American Society of Agronomy, Journal	Subscription
American Thresherman & Farm Power	Complimentary
Analyst	Subscription
Annales de L'Institut Pasteur	Subscription
Annals and Magazine of Natural History	Subscription
Annals of Applied Biology	Subscription
Annals of Botany	Subscription
Annals of the Missouri Botanical Garden	Subscription
Better Fruit	Complimentary
Biological Bulletin	Subscription
Boletim de Agricultura	Complimentary
Boletim do Instituto Agronomico	Complimentary
Boletin Agricola, Panama	Complimentary
Boletin do la Sociedad Nacional de Agricultura	Complimentary
Botanical Gazette	Subscription
Bulletin de I'Institut Pasteur	Subscription
Bulletin fuer angewandte Botanik	Complimentary
Caledonia Era	Complimentary
California Academy of Sciences, Proceedings	Complimentary

California Cultivator	Complimentary
California Fruit News	Subscription
California University Publications — Agricultural Sciences, Botany and Zoology	Complimentary
Canadian Entomologist	Subscription
Canadian Horticulturist	Complimentary
Chemical Abstracts	Subscription
Chemical Society, Journal	Subscription
Chicago Dairy Produce	Complimentary
Cold	Complimentary
Colonial Dairy Produce Report	Complimentary
Columbus Horticultural Society Journal	Complimentary
Commercial Fertilizer	Complimentary
Commercial Poultry	Complimentary
Country Gentleman	Subscription
Country Life in America	Subscription
Creamery and Milk Plant Monthly	Complimentary
Dairy and Produce Review	Complimentary
Duroc Bulletin	Complimentary
Entomological News	Subscription
Entomological Society of America, Annals	Subscription
Entomological Society of Washington, Proceedings	Subscription
Entomologist	Subscription
Entomologist's Record	Subscription
Farm and Dairy and Rural Home	Complimentary
Farm and Fireside	Complimentary
Farm Engineering	Complimentary
Farm, Stock and Home	Complimentary
Farmers' Advocate	Complimentary
Farmers' Review	Complimentary
Feathered World	Subscription
Field, The, Illustrated	Complimentary
Finnska Mosskulturforeningens Arsbok	Complimentary
Florists' Exchange	Subscription
Flour and Feed	Complimentary
Fruit Grower	Complimentary
Fruit and Produce Marketer	Complimentary
Garden	Subscription
Gardeners' Chronicle	Subscription
Gas and Oil Power	Complimentary
Gas Power	Complimentary
Gleanings in Bee Culture	Complimentary
Grape Belt, The	Complimentary
Green's Fruit Grower	Complimentary
Guide to Nature	Subscription
Harvester World	Complimentary
Hawaiian Forester and Agriculturist	Complimentary

Herd Register	Complimentary
Hoard's Dairyman	Complimentary
Homestead	Complimentary
Hospodar	Complimentary
Insect World (Japanese)	Complimentary
International Garden Club, Journal	Subscription
Journal of Agricultural Research	Complimentary
Journal of Agricultural Science	Subscription
Journal of Agriculture, Victoria	Complimentary
Journal of American Medical Association	Subscription
Journal of Association of Official Agricultural Chemists	Subscription
Journal of Biological Chemistry	Subscription
Journal of Board of Agriculture (English)	Complimentary
Journal of the College of Agriculture, Tokyo	Complimentary
Journal of The Department of Agriculture of South Australia	Complimentary
Journal of the Department of Agriculture of Victoria	Complimentary
Journal of the New Zealand Department of Agriculture	Complimentary
Journal of Economic Biology	Subscription
Journal of Experimental Medicine	Subscription
Journal of Experimental Zoology	Subscription
Journal of Genetics	Subscription
Journal of Heredity	Subscription
Journal of Home Economics	Subscription
Journal of Hygiene	Subscription
Journal of Industrial and Engineering Chemistry	Subscription
Journal of Pathology and Bacteriology	Subscription
Journal of Physiology	Subscription
Market Growers' Journal	Complimentary
Memoirs of the Department of Agriculture in India	Complimentary
Michigan Farmer	Complimentary
Monthly Bulletin, International Institute of Agriculture	Complimentary
Monthly Bulletin of the N. Y. State Department of Health	Complimentary
Monthly Weather Review	Complimentary
Mycologia	Subscription
National Farmer	Complimentary
National Farmer and Stockgrower	Complimentary
National Nurseryman	Complimentary
Naturaliste Canadienne	Complimentary
New York Academy of Science, Annals and Transactions	Subscription
New York Botanical Garden, Bulletin	Complimentary
New York Entomological Society, Journal	Subscription
New York Fruit & Produce News	Complimentary
Nut Grower	Complimentary
Ohio Journal of Science	Subscription
Pacific Coast Fanciers' Monthly	Subscription
Parasitology	Subscription
Photo-Miniature	Subscription

Physiological Researches	Subscription
Phytopathology	Subscription
Poultry Herald	Subscription
Power and Engineer	Subscription
Progressive Farmer	Complimentary
Psyche	Subscription
Quarterly Journal	Complimentary
Recreation	Subscription
Reclamation Record	Complimentary
Reliable Poultry Journal	Subscription
Review of Applied Entomology	Subscription
Revista Industrial y Agricola de Tucuman	Complimentary
Revue de Viticulture (For Vineyard Laboratory, Fredonia, N. Y.)	Subscription
Revue Generale de Botanique	Subscription
Revue Horticole	Subscription
Rhode Island Red Journal	Complimentary
Royal Agricultural Society Journal	Subscription
Rural Life	Complimentary
Rural New Yorker	Subscription
Science	Subscription
Scientific American	Subscription
Seed World	Complimentary
Societe Entomologique de France, Bulletin	Complimentary
Soil Science	Subscription
Southern Planter	Complimentary
Stazione Sperimentale Agrarie Italiane	Complimentary
Torrey Botanical Club, Bulletins and Memoirs	Subscription
Utica Semi-Weekly Press	Complimentary
Vertical Farming	Complimentary
Wallace's Farmer	Complimentary
Wilson Bulletin	Complimentary

METEOROLOGICAL RECORDS FOR 1917.

METEOROLOGICAL RECORDS FOR 1917.

READING OF THE STANDARD AIR THERMOMETER.

DATE.	JANUARY.			FEBRUARY.			MARCH.			APRIL.			MAY.			JUNE.		
	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.
1	18.	32.	36.	27.	21.	16.	24.	30.	31.	60.	70.	57.	49.	52.	48.	60.	70.	74.
2	35.	36.	33.	5.	6.	—3.	20.	29.	29.	56.	56.	60.	38.	40.	44.	53.	64.	67.
3	34.	38.	37.	—3.	6.	9.	29.	35.	33.	34.	45.	45.	35.	40.	43.	63.	60.	56.
4	34.	43.	38.	12.	24.	24.	24.	21.	18.	39.	49.	50.	39.	44.	45.	67.	79.	77.
5	38.	40.	44.	4.	9.	11.	17.	24.	19.	43.	45.	40.	37.	39.	40.	62.	63.	60.
6	30.	31.	32.	14.	20.	19.	13.	23.	27.	34.	39.	34.	40.	47.	48.	51.	60.	62.
7	32.	38.	32.	21.	29.	34.	24.	37.	38.	31.	33.	37.	41.	51.	51.	61.	66.	60.
8	28.	43.	42.	30.	35.	35.	38.	41.	34.	32.	35.	32.	43.	56.	48.	59.	70.	70.
9	37.	40.	37.	15.	17.	9.	30.	32.	30.	25.	29.	27.	48.	55.	58.	65.	70.	72.
10	30.	18.	12.	—2.	8.	8.	27.	36.	37.	28.	34.	39.	42.	48.	53.	63.	68.	64.
11	2.	5.	3.	2.	6.	5.	38.	42.	40.	32.	50.	54.	39.	53.	43.	57.	62.	66.
12	3.	13.	12.	—4.	4.	1.	29.	32.	29.	48.	57.	44.	42.	50.	54.	58.	73.	77.
13	21.	26.	25.	—4.	14.	18.	22.	31.	32.	29.	31.	34.	50.	55.	58.	69.	79.	82.
14	24.	17.	12.	18.	25.	24.	33.	34.	35.	30.	35.	36.	49.	59.	62.	59.	72.	73.
15	12.	18.	17.	14.	29.	22.	31.	33.	30.	33.	33.	37.	40.	49.	52.	49.	54.	52.
16	12.	21.	19.	16.	26.	26.	31.	34.	37.	32.	38.	38.	46.	47.	52.	51.	57.	62.
17	13.	24.	25.	22.	42.	45.	37.	38.	35.	37.	52.	56.	43.	56.	54.	56.	67.	72.
18	25.	28.	25.	20.	22.	22.	26.	29.	24.	47.	69.	66.	46.	67.	75.	56.	70.	68.
19	15.	20.	10.	14.	23.	27.	12.	19.	21.	50.	63.	68.	55.	74.	78.	61.	75.	83.
20	19.	29.	27.	37.	30.	24.	31.	49.	42.	56.	65.	73.	55.	56.	59.	71.	62.	68.
21	12.	16.	15.	17.	23.	23.	30.	40.	37.	57.	59.	55.	44.	51.	46.	57.	68.	71.
22	35.	29.	16.	20.	27.	30.	32.	43.	45.	48.	69.	67.	44.	54.	53.	69.	79.	76.
23	14.	27.	26.	34.	37.	39.	39.	53.	43.	35.	45.	52.	49.	48.	47.	57.	71.	63.
24	19.	31.	31.	34.	26.	25.	37.	46.	43.	37.	47.	43.	43.	44.	41.	53.	57.	67.
25	27.	28.	18.	12.	21.	25.	39.	59.	59.	38.	52.	56.	42.	54.	55.	62.	73.	75.
26	13.	17.	14.	39.	48.	46.	42.	60.	58.	46.	54.	41.	53.	65.	65.	60.	68.	71.
27	9.	12.	19.	30.	32.	30.	49.	54.	35.	40.	45.	43.	50.	58.	59.	71.	75.	74.
28	32.	26.	24.	22.	26.	34.	31.	39.	39.	39.	49.	48.	45.	53.	51.	63.	72.	70.
29	30.	38.	39.	....	....	....	41.	40.	44.	44.	58.	56.	49.	50.	48.	62.	73.	64.
30	33.	38.	36.	....	....	....	43.	43.	45.	48.	52.	54.	51.	64.	70.	63.	69.	74.
31	29.	32.	30.	....	....	....	37.	53.	67.	....	....	....	58.	74.	69.	....	....	....
Averages	23.0	27.5	25.3	16.6	22.7	22.6	30.4	38.3	36.4	40.2	48.6	48.0	45.3	53.3	53.8	60.1	68.2	69.0

Reading of the Standard Air Thermometer (*concluded*).

DATE.	July.			August.			September.			October.			November.			December.		
	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.	7 A. M.	12 M.	5 P. M.
1	63.	63.	74.	84.	94.	96.	65.	78.	82.	46.	49.	50.	27.	36.	35.	37.	38.	35.
2	69.	79.	79.	83.	84.	84.	71.	72.	69.	43.	60.	59.	26.	43.	39.	25.	23.	22.
3	66.	76.	70.	69.	79.	72.	59.	69.	65.	52.	59.	62.	29.	41.	40.	24.	29.	29.
4	65.	69.	74.	64.	74.	77.	55.	63.	66.	50.	53.	55.	32.	47.	34.	26.	34.	33.
5	61.	72.	74.	66.	81.	82.	55.	65.	66.	50.	59.	58.	24.	42.	40.	31.	31.	26.
6	62.	76.	79.	69.	84.	83.	59.	67.	63.	43.	44.	45.	33.	53.	40.	18.	24.	21.
7	69.	80.	82.	68.	81.	86.	55.	61.	58.	39.	52.	52.	31.	35.	33.	16.	21.	17.
8	62.	68.	67.	74.	78.	78.	53.	63.	64.	50.	43.	45.	28.	43.	41.	14.	20.	15.
9	63.	67.	73.	66.	75.	71.	57.	69.	64.	34.	49.	52.	33.	46.	42.	13.	13.	8.
10	63.	65.	66.	61.	74.	77.	46.	51.	48.	32.	47.	45.	40.	47.	43.	6.	14.	13.
11	61.	67.	68.	66.	74.	78.	41.	55.	57.	38.	51.	50.	33.	52.	50.	9.	16.	7.
12	61.	73.	67.	67.	79.	78.	50.	66.	69.	45.	50.	47.	39.	37.	34.	6.	19.	15.
13	66.	75.	74.	66.	82.	64.	52.	69.	71.	40.	47.	46.	22.	40.	35.	16.	24.	24.
14	63.	77.	76.	64.	73.	74.	54.	74.	74.	37.	55.	56.	25.	48.	42.	23.	18.	17.
15	69.	73.	79.	69.	82.	76.	55.	73.	70.	51.	54.	53.	32.	38.	39.	10.	13.	10.
16	68.	78.	74.	67.	80.	74.	56.	66.	70.	44.	53.	49.	35.	37.	36.	5.	14.	14.
17	65.	75.	74.	63.	70.	65.	55.	63.	70.	34.	49.	48.	38.	47.	50.	12.	24.	20.
18	67.	81.	75.	60.	72.	71.	58.	74.	73.	50.	63.	60.	42.	55.	39.	15.	29.	28.
19	70.	79.	81.	67.	80.	82.	59.	75.	74.	62.	63.	40.	25.	30.	30.	33.	37.	36.
20	69.	85.	81.	68.	86.	81.	61.	70.	71.	35.	45.	41.	34.	41.	41.	37.	41.	36.
21	73.	72.	79.	63.	70.	75.	55.	61.	60.	33.	49.	45.	35.	41.	40.	38.	42.	38.
22	73.	86.	78.	63.	75.	78.	51.	60.	55.	37.	47.	42.	43.	49.	47.	19.	22.	18.
23	76.	88.	89.	68.	79.	75.	48.	61.	62.	37.	41.	42.	28.	28.	25.	11.	27.	31.
24	69.	86.	88.	69.	80.	80.	49.	63.	61.	40.	36.	40.	23.	22.	20.	35.	40.	43.
25	74.	88.	90.	64.	62.	58.	49.	67.	67.	35.	41.	42.	15.	20.	19.	24.	23.	20.
26	77.	85.	75.	57.	72.	75.	49.	68.	69.	33.	50.	50.	19.	24.	19.	16.	23.	12.
27	76.	76.	76.	62.	83.	85.	55.	74.	71.	43.	53.	51.	11.	22.	23.	11.	20.	21.
28	68.	79.	84.	66.	67.	64.	56.	63.	59.	37.	47.	49.	28.	29.	33.	24.	10.	—1.
29	66.	76.	91.	55.	64.	63.	51.	70.	64.	41.	54.	55.	30.	39.	36.	—11.	—5.	—7.
30	82.	92.	92.	58.	61.	69.	52.	59.	57.	47.	40.	37.	35.	39.	40.	—15.	—1.	—5.
31	84.	93.	96.	57.	71.	73.	....	....	....	31.	32.	35.	....	....	....	—8.	2.	0.
Averages	68.2	77.2	78.2	65.9	76.3	75.6	54.4	66.3	65.6	41.5	49.5	49.3	30.1	39.0	39.5	16.7	22.0	19.2

READING OF MAXIMUM AND MINIMUM THERMOMETERS FOR 1917.

DATE.	JANUARY.		FEBRUARY.		MARCH.		APRIL.		MAY.		JUNE.	
	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.	5 P. M. Max.	5 P. M. Min.
1	37.	12.	32.	16.	35.	20.	71.	50.	55.	45.	74.	57.
2	37.	33.	16.	3.	32.	19.	66.	50.	48.	37.	74.	52.
3	40.	26.	10.	—5.	36.	27.	66.	31.	45.	33.	67.	55.
4	45.	34.	25.	8.	33.	17.	54.	32.	46.	32.	80.	47.
5	44.	31.	24.	1.	26.	16.	54.	38.	45.	36.	79.	54.
6	47.	27.	22.	3.	29.	11.	41.	32.	50.	37.	62.	49.
7	40.	23.	35.	12.	40.	14.	37.	30.	53.	35.	75.	49.
8	44.	20.	36.	29.	41.	33.	37.	32.	57.	39.	74.	48.
9	43.	36.	36.	9.	34.	29.	32.	22.	60.	42.	74.	51.
10	37.	12.	10.	—3.	41.	21.	39.	23.	58.	37.	72.	59.
11	17.	1.	8.	—4.	46.	34.	59.	28.	55.	37.	69.	52.
12	15.	1.	5.	—5.	42.	26.	54.	42.	56.	38.	78.	49.
13	28.	6.	20.	—8.	34.	19.	44.	28.	59.	41.	83.	59.
14	36.	12.	27.	15.	37.	22.	38.	29.	63.	42.	82.	58.
15	18.	7.	30.	9.	37.	26.	40.	31.	63.	37.	73.	48.
16	22.	8.	28.	7.	41.	27.	39.	30.	53.	42.	63.	46.
17	28.	11.	46.	15.	39.	33.	50.	29.	57.	38.	72.	46.
18	28.	19.	45.	19.	37.	22.	71.	37.	75.	36.	72.	50.
19	25.	9.	28.	12.	24.	11.	72.	48.	82.	43.	83.	57.
20	33.	6.	41.	23.	50.	13.	78.	52.	78.	53.	83.	58.
21	28.	11.	26.	15.	43.	26.	75.	53.	61.	40.	75.	53.
22	35.	9.	31.	17.	47.	25.	80.	37.	58.	38.	80.	57.
23	30.	11.	40.	17.	55.	33.	60.	34.	53.	38.	76.	51.
24	35.	15.	39.	23.	47.	35.	54.	31.	47.	38.	68.	52.
25	31.	17.	25.	8.	63.	30.	61.	25.	58.	39.	75.	57.
26	18.	10.	55.	25.	65.	39.	62.	43.	71.	43.	76.	55.
27	19.	8.	46.	29.	58.	35.	63.	36.	67.	46.	76.	60.
28	33.	18.	35.	21.	39.	29.	51.	34.	59.	43.	75.	54.
29	40.	19.	......	......	44.	33.	63.	30.	54.	47.	75.	59.
30	41.	32.	......	......	45.	30.	57.	44.	72.	39.	74.	55.
31	37.	27.	......	......	68.	31.	......	......	74.	51.	......	......
Averages	32.6	16.5	29.3	11.1	42.1	25.4	56.2	35.3	59.	40.	74.6	53.2

Reading of Maximum and Minimum Thermometers for 1917 *(concluded)*.

DATE.	July.		August.		September.		October.		November.		December.	
	5 p. m. Max.	5 p. m. Min.	5 p. m. Max.	5 p. m. Min.	5 p. m. Max.	5 p. m. Min.	5 p. m. Max.	5 p. m. Min.	5 p. m. Max.	5 p. m. Min.	5 p. m. Max.	5 p. m. Min.
1	75.	53.	96.	76.	82.	64.	57.	45.	43.	25.	42.	34.
2	80.	53.	96.	74.	82.	62.	64.	36.	43.	25.	36.	22.
3	79.	52.	95.	60.	70.	52.	62.	47.	47.	33.	31.	21.
4	76.	50.	78.	56.	70.	58.	62.	48.	42.	27.	34.	24.
5	80.	51.	84.	52.	69.	44.	63.	47.	51.	22.	33.	26.
6	82.	53.	85.	66.	67.	57.	58.	41.	54.	27.	26.	17.
7	82.	57.	86.	57.	66.	46.	54.	35.	42.	30.	24.	15.
8	82.	60.	86.	69.	68.	49.	52.	42.	47.	27.	20.	8.
9	74.	60.	78.	65.	72.	49.	50.	32.	55.	32.	13.	8.
10	73.	62.	77.	56.	64.	42.	50.	26.	49.	36.	15.	5.
11	69.	58.	80.	54.	57.	41.	55.	35.	58.	30.	19.	7.
12	74.	54.	83.	51.	71.	40.	52.	44.	52.	33.	22.	—1.
13	77.	55.	84.	55.	75.	42.	49.	36.	44.	22.	25.	7.
14	77.	58.	78.	62.	78.	46.	59.	34.	52.	24.	24.	17.
15	80.	60.	84.	64.	75.	51.	56.	50.	42.	28.	20.	9.
16	80.	58.	81.	58.	74.	52.	59.	39.	40.	34.	14.	2.
17	76.	60.	75.	59.	74.	52.	54.	31.	54.	35.	26.	9.
18	82.	62.	72.	55.	77.	49.	64.	44.	52.	34.	32.	15.
19	81.	69.	85.	57.	80.	55.	64.	40.	39.	25.	37.	26.
20	87.	59.	90.	65.	75.	58.	48.	31.	42.	25.	42.	35.
21	84.	63.	86.	63.	72.	54.	50.	29.	43.	33.	42.	34.
22	90.	68.	80.	59.	62.	46.	50.	35.	51.	39.	41.	18.
23	91.	65.	80.	65.	66.	35.	46.	39.	47.	28.	34.	9.
24	90.	69.	83.	66.	66.	42.	42.	34.	25.	20.	44.	30.
25	91.	66.	80.	58.	74.	41.	42.	34.	24.	14.	43.	20.
26	90.	69.	76.	48.	74.	44.	54.	32.	28.	16.	25.	12.
27	82.	70.	87.	53.	79.	51.	54.	43.	24.	9.	23.	5.
28	84.	64.	85.	60.	71.	51.	52.	35.	33.	23.	28.	—1.
29	91.	65.	67.	55.	72.	45.	62.	35.	41.	30.	—1.	—12.
30	94.	78.	69.	57.	64.	51.	64.	36.	41.	30.	3.	—18.
31	96.	79.	75.	47.	......	......	37.	30.	......	......	5.	—15.
Averages	82.2	61.3	81.6	59.4	71.5	48.6	54.3	37.5	43.5	27.3	26.5	12.5

SUMMARY OF AVERAGES OF MAXIMUM, MINIMUM AND STANDARD AIR THERMOMETERS FOR 1917.

	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Maximum	32.6	29.3	42.1	42.2	59.0	74.6	82.2	81.6	71.5	54.3	43.5	26.5
Minimum	16.5	11.1	25.3	25.4	40.0	53.2	61.3	59.4	48.6	37.5	27.3	12.5
Standard 7 A. M.	23.0	16.6	30.4	40.2	45.3	60.1	68.2	65.9	54.4	41.5	30.1	16.7
Standard 12 M.	27.5	22.7	38.0	48.6	53.3	68.2	77.2	76.3	66.3	49.5	39.0	22.0
Standard 5 P. M.	25.3	22.6	36.4	38.0	53.8	69.0	78.2	75.6	65.6	49.3	39.5	19.2

MONTHLY MAXIMUM AND MINIMUM TEMPERATURES FROM 1883 TO 1917 INCLUSIVE.

(Highest and Lowest Record for Each Month in Heavy Type.)

	JANUARY.				FEBRUARY.				MARCH.				APRIL.			
	MAX.		MIN.		MAX.		MIN.		MAX.		MIN.		MAX.		MIN.	
	Date.	Temp.	Date	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.
1883	18	44.	11	—9.	17	48.	24	—2.	19	61.	9	2.	16	75.	1	19.
1884	14	42.	26	—13.	7	55.	29	—3.	30	54.	1	—4.	28	74.	1	23.
1885	1	61.	29	—6.	10	38.	11	—11.5	28	48.	13	**—11.**	24	84.5	10	20.5
1886	5	52.5	13	**—18.7**	9	50.	27	—11.	16	58.	2	—2.5	24	80.5	4	22.
1887	24	50.7	19	—8.	9	54.2	27	—4.	3	51.7	1 & 5	8.7	11	75.7	1	17.2
1888	2	43.2	23	—6.	21	49.	10	—7.	28	57.	13	0.	29	82.5	8	19.
1889	18	55.	20	5.	23	42.	4 & 24	—7.	28	61.8	30	18.5	20	84.	1	26.
1890	6	67.	29	9.	5	64.5	11&21	9.5	13	62.	8	2.	13	78.8	1& 19	23.
1891	3	46.	17	4.	26	56.8	15	2.5	12	57.	2	4.5	28	81.4	7	21.
1892	3	48.	10	—5.	15	44.	6	2.8	27	52.2	4	6.	6	78.	17	25.5
1893	29	46.	11	—6.	15	47.4	5	—1.	24	54.	5	9.	13	75.3	26.2	25.
1894	5	59.	13	11.	20	47.6	27	—8.5	18	73.	26	15.	21	71.3	2	20.
1895*	7	45.	19	4.	25	46.	8	—14.	25	52.	5 & 16	12.	30	80.	3	28.
1896	30	44.	6	—16.5	29	49.	17	**—21.**	31	56.5	24	—2	17	87.	4& 5	19.
1897	5	58.	20	—3.5	18	49.5	1 & 27	5.5	21	64.	1	—1.	26	82.	20	19.
1898	13	57.	30&31	—4.	12	56.5	2 & 3	—2.	11	†65.	2	17.5	14&18	69.	69	18.
1899	5	59.	12	—4.	21	52.5	11	—8.	73	63.	21	13.	30	82.	3	23.
1900	23	56.	1	2.	14	57.	27	0.	10	46.	12	—3.	30	‡73.5	9	22.
1901	16	48.	20	—2.	16	36.	24	—2.5	24	67.	6	—1.	28	78.	12	28.
1902	3	44.	28	2.	28	52.	6	—3.	12	66.5	19	14.	22	87.	5	25.
1903	3	48.	9	—2.	28	62.5	18	—4	19	78.5	1 & 2	19.	30	86.	5	21.
1904	23	48.	19	—14.	7	58.	16	18.	26	58.	4	8.	24	67.5	14	16.
1905	1	49.	26	—2.	20	45.	5 & 14	—6.	29	82.	5	1.	27&28	75.	16	23.
1906	21	**71.**	9	4.	24	64.	6 & 7	—7.	27	51.	25	2.	19	74.	2	25.
1907	6	53.	24	—18.	2	47.	12	—4.	29	**83.**	7	—1.	29	73.	2	19.
1908	22	45.	31	—9.	15	53.	2 & 5	—14.	28	73.	1	8.	27	78.	4	18.
1909	24	64.	19	—7.	5	52.	1	—1.	10	52.	5	5.	19	75.	11	**12.**
1910	2 & 22	45.	5	—8.	16	29.	7	—3.	24	82.	14&18	17.	4	84.	7	27.
1911	27&30	48.	5	—1.	17	52.	22	4.	27	60.	16	—1.	29	80.	3	18.
1912	18	44.	14	—12.	24	48.	10	—10.	31	62.	4	—1.	6	78.	4	19.
1913	17	57.	13	8.	20	**65.**	10	—10.	14&15	69.	7 & 8	1.	24	84.	20	24.
1914	29	51.	13&14	—9.	3	52.	13&24	—14.	26	65.	12	7.	19	82.	9 & 13	22.
1915	7	46.	30	—3.	21&23	53.	10	10.	25	50.	1	11.	25&26	**88.**	5	23.
1916	27	61.	17	—3.	1	47.	15	—8.	31	58.	18	—3.	21	73.	8	26.
1917	6	47.	11&12	1.	26	55.	13	—8.	31	68.	6 & 29	11.	22	80.	11&18	23.

* Data from record kept by Mr. Edgar Parker for the year 1895; Station record not available.
† Maximum for first eleven days only. Record incomplete.
‡ Thermometers broken. Record not taken from April 19th to 24th inclusive.

MONTHLY MAXIMUM AND MINIMUM TEMPERATURES FROM 1883 TO 1917 INCLUSIVE (*continued*).

(Highest and Lowest Record for Each Month in Heavy Type.)

	MAY.				JUNE.				JULY.				AUGUST.			
	MAX.		MIN.		MAX.		MIN.		MAX.		MIN.		MAX.		MIN.	
	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.
1883	11	87.	1 & 14	31.	7	86.5	2	42.	5	89.	1	46.	23	92.	15	46.
1884	24	88.	30	32.	25	90.	15	41.	2	87.5	15	50.	20	95.	25	44.
1885	18	81.7	3	27.5	14	86.5	23	41.5	18	90.5	12	46.5	1	89.	28	45.
1886	23	79.5	17&18	37.2	14	86.2	1	42.2	7	95.	1	45.2	30	91.5	6	47.7
1887	23	88.2	14	37.5	17	89.2	15	47.7	3	95.5	11	58.7	3	88.5	8	46.
1888	13	79.8	3	29.	23	94.1	4	40.	5	89.8	16	47.	9	92.6	23	48.3
1889	18	91.8	29	32.	22	85.	5	46.	11	90.7	6	50.5	31	86.7	16&17	50.3
1890	4	80.7	2	30.	30	85.6	8	44.8	9	94.5	24	46.5	4	96.2	24	46.0
1891	11	85.5	4	29.5	16	95.	6	40.	14	92.	31	46.	12	92.	29	46.5
1892	31	78.	9	34.2	14	92.	11	45.8	29	96.3	2	46.4	10	95.5	28	49.
1893	25	88.	9	35.	21	94.	1	44.	26	95.5	24	48.4	11	94.5	13	49.
1894	2	85.4	14	32.6	23	91.6	6	39.	21	97.	10	49.6	25	93.	27	45.3
1895*	31	92.	13&21	36.	3	**96.**	7	54.	8	94.	11	52.	11	88.	22	44.
1896	11	87.5	7 & 20	40.	21	89.	3	41.	3	94.	18	49.	6 & 7	96.	29	44.
1897	24	80.	8	32.5	24&25	87.5	2	42.	11	97.	15	57.	15	87.5	21	46.
1898	29	79.	6	34.	9	90.	16	40.	4	96.5	12	**40.**	24	90.5	28	47.
1899	2	87.5	15	32.5	6 & 24	93.	11	41.5	4	97.5	1	50.	20	97.5	15	44.5
1900	15&16	88.5	7	27.	25	93.	10	45.	17	96.	1	50.	11	97.	2	51.
1901	23	78.	16	36.	27, 28 & 29	95.5	2	42.	1	97.5	20	54.5	22	90.	5	52.
1902	22	90.	11	26.	3	85.	6	38.	14&27	90.	1	53.	31	90.	13	47.
1903	19	89.	2	**24.**	30	86.5	1	39.	9	94.	15	50.	18	85.5	8 & 14	45.
1904	25	88.	12	31.5	5–24 & 25	89.	12&17	45.	19	93.	3	49.	25	89.5	19	45.
1905	3	82.	2	29.5	19	90.	1	40.	18	92.	22	48.5	10	93.	27	**41.**
1906	24	88.5	11&21	30.	8	92.	12	37.	20–22 & 23	89.	25	50.	5	93.	16	47.
1907	14	85.	2–11 & 12	28.	18	94.	3	41.	16	90.	4	46.	12	96.5	19	41.5
1908	29	90.	1–4&5	31.	19	92.	12	43.	6–11 & 30	94.	9	52.	4	95.	25	46.
1909	31	78.	2 & 3	33.	28	90.	8	43.	15	92.	4	42.	8	98.	31	42.
1910	20	79.	16	31.5	22	89.9	4	**36.**	9	96.5	5	50.	3 & 15	90.	27	44.
1911	22	**97.**	3	27.	11	90.5	17	46.	5	**105.0**	25–26 & 27	50.	8	94.5	30	47.
1912	24	88.	14	34.	1	89.	8	40.	8 & 10	95.	1	41.	14	92.	17	44.
1913	4	91.	11	30.	30	92.	9	37.	1 & 4	95.	12	50.	17	98.	25&26	44.
1914	26&27	90.	1	34.	24&25	90.	20	39.	11	93.	4	50.	9	94.	26	47.
1915	23	77.	3 & 4 10&27	34.	18&19	86.	4 & 9	41.	31	90.	22&23	49.	1	89.	27	43.
1916	24&28	82.	10&19	37.	27	82.	1	41.	30	96.	1	49.	22	**101.**	2&29	46.
1917	19	82.	4	32.	13&19 & 20	83.	16&17	46.	31	96.	4	50.	1 & 2	96.	31	47.

* Data from record kept by Mr. Edgar Parker for the year 1895; Station record not available.

MONTHLY MAXIMUM AND MINIMUM TEMPERATURES FROM 1883 TO 1917, INCLUSIVE (*concluded*).

(Highest and Lowest Record for Each Month in Heavy Type.)

	SEPTEMBER.				OCTOBER.				NOVEMBER.				DECEMBER.			
	MAX.		MIN.		MAX.		MIN.		MAX.		MIN.		MAX.		MIN.	
	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.	Date.	Temp.
1883	17	80.	11	37.	11	78.	17&18	25.	22	70.	17	13.	9 & 14	56.	23	—7.5
1884	5	94.	14	**26.**	5	84.2	27	23.	11	63.	25	15.	31	55.5	20	—15.5
1885	27	83.7	24	40.	1	79.	13	25.	8 & 13	68.	28	18.	24	53.	9	4.
1886	11	89.5	22	49.	10	76.7	**17**	27.5	3	68.2	28	17.	11&25	46.	6	—6.
1887	22	81.7	27	37.2	9	78.5	31	21.2	28	68.	30	15.	12	54.7	2	—3.
1888	1 & 10	83.	7	40.	6	62.7	22	29.	1 & 3	73.	23	**8.**	27	53.	22	4.
1889	4	84.	22 & 23&29	40.	2	68.7	24	21.2	4	61.7	17	17.8	25	60.5	4 & 5	8.
1890	8	83.6	25	35.5	5	69.8	31	32.	8	65.4	28	17.	1	46.2	20	3.
1891	26	92.8	30	43.	4	**89.4**	12&25	27.	1	68.	29	12.	5	57.7	18	7.
1892	26	88.	20	39.	1	82.	2	33.1	19	60.	24	18.	9	49.2	27	—3.7
1893	5	80.	26	37.4	13	76.	31	25.	3	62.	27	19.	26	62.	14	1.5
1894	4	90.	26	33.	1	76.5	15	33.	3	65.	29	12.	17	59.	29	—0.2
1895*	4	94.	15&30	42.	2	72.	30	28.	7	68.	21	19.	20&21	62.	13	—2.
1896	12	95.	23	36.	30	77.5	10&19	29.	19	70.	21	19.5	14	58.	28	2.
1897	11	**98.**	21	37.5	16	88.	10&18	30.	6	65.	24	16.5	12	61.5	24	2.
1898	4	94.	21	40.5	1	85.5	28	31.	5	63.	28	16.	31	54.	14	3.
1899	4	92.	15&30	30.	15	86.	3	26.	19	60.	14	25.	12	60.	31	—1.
1900	12	95.	19	37.	6 & 7	89.	20	28.	22	70.	17	19.	4	55.	10&14	4.
1901	6	89.	26	36.	10&11	74.	28	28.	1	65.	27	13.	14	62.	18	—1.
1902	1	90.	15	38.	19	74.	10 & 22&30	29.	14	73.	29	22.	2	52.	9	—5.
1903	14	90.	29	35.	1	73.	25&27	28.	4	70.	26&27	12.	3	46.	19	—4.
1904	3	88.	23	33.	10	81.	31	22.	3	65.	29	9.	23	53.	16	—2.
1905	30	88.5	26	36.	1	85.	26	**20.5**	12	61.	14	11.	29	52.5	15	1.
1906	18	91.5	25	38.	5	79.5	13&31	30.	19	62.	30	16.	6	52.	8	—1.
1907	20	90.	27	39.	†3	80.	31	24.	1	59.	12&16	22.	30	57.	22	13.5
1908	10	92.	30	37.	18	83.	21	27.	26	68.	5	18.	1	64.	23	3.
1909	14	93.	2 & 6	35.	9	82.5	29&30	27.	11	**75.**	24	21.	6	45.	30	1.
1910	6	87.	15&23	40.	6	81.	30	26.	1	59.	23	21.	29	41.	31	—2.5
1911	2	87.	14	35.	4	78.	3	33.	11	68.	13	18.	9	**67.9**	4 & 30	13.
1912	6 & 10	95.	30	34.	6	83.	16	31.	6 & 7	69.	28	20.	6	65.	9 & 12	12.
1913	3	95.	15	28.	6	81.	31	29.	22	73.	27	22.	7	56.	28	6.
1914	22	92.	29	38.	10	84.	27	26.	1	69.	24	16.	2	64.	26&27	—6.
1915	14	93.	28	35.	13	75.	25	29.	1	68.	18	21.	25	45.	31	4.
1916	7	93.	30	39.	8	87.	27	29.	8	74.	25	16.	5	62.	30	4.
1917	1 & 2	82.	23	35.	2 & 18 19&30	64.	10	26.	11	58.	27	9.	24	44.	30	—**18.**

* Data from record kept by Mr. Edgar Parker for the year 1895; Station record not available.

† Thermometer broken on the 27th, 28th, and 29th of October.

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YEARLY MAXIMUM AND MINIMUM TEMPERATURES FROM 1883 TO 1917 INCLUSIVE.

(Highest and Lowest Record for THE TIME in Heavy Type.)

	MAXIMUM FOR EACH YEAR.		MINIMUM FOR EACH YEAR.	
	Date.	Temp.	Date.	Temp.
1883	Aug. 23	92.	Jan. 11	— 9.
1884	Aug. 20	95.	Dec. 20	—15.5
1885	July 18	90.5	Feb. 11	—11.5
1886	July 7	95.	Jan. 13	—18.7
1887	July 3	95.5	Jan. 19	— 8.
1888	June 23	94.1	Feb. 10	— 7.
1889	May 18	91.8	Feb. 4 and 24	— 7.
1890	Aug. 4	96.2	Mar. 8	2.
1891	June 16	95.	Feb. 15	2.5
1892	July 29	96.3	Jan. 10	— 5.
1893	July 26	95.5	Jan. 11	— 6.
1894	July 21	97.	Feb. 27	— 8.5
1895*	June 3	96.	Feb. 8	—14.
1896	Aug. 6 and 7	96.	Feb. 17	**—21.**
1897	Sept. 11	98.	Jan. 20	— 3.5
1898	July 4	96.5	Jan. 30 and 31	— 4.
1899	July 4 and Aug. 20.	97.5	Feb. 11	— 8.
1900	Aug. 1	97.	Feb. 27	0.
1901	July 1	97.5	Feb. 24	2.5
1902	May 24, July 14 and 27, August 31 and Sept. 1	90.	Dec. 9	— 5.
1903	July 9	94.	Feb. 18 and Dec. 19.	— 4.
1904	July 19	93.	Feb. 16	—18.
1905	Aug. 10	93.	Feb. 5 and 14	— 6.
1906	Aug. 5	93.	Feb. 6 and 7	— 7.
1907	Aug. 12	96.5	Jan. 24	—18.
1908	Aug. 4	95.	Jan. 2 and 5	—14.
1909	Aug. 8	98.	Jan. 19	— 7.
1910	July 9	96.5	Jan. 5	— 8.
1911	July 5	**105.**	Jan. 5	— 1.
1912	Sept. 6	95.	Jan. 14	—12.
1913	Aug. 17	98.	Feb. 10	—10.
1914	Aug. 9	94.	Feb. 13 and 24	—14.
1915	Sept. 14	93.	Jan. 30	— 3.
1916	Aug. 22	101.	Feb. 15	— 8.
1917	July 31, Aug. 1 & 2.	96.	Dec. 30	—18.

* Data from record kept by Mr. Edgar Parker; Station record not available.

MONTHLY AND YEARLY MEANS OF TEMPERATURES SINCE 1882.

YEAR.	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Dec.	Yearly averages.
1883	17.4	22.3	23.6	43.3	52.0	66.6	67.4	65.6	56.3	46.6	39.1	27.5	44.0
1884	17.6	28.3	29.5	40.7	54.3	67.1	66.5	69.9	65.2	50.5	36.5	27.2	46.1
1885	20.6	11.4	18.8	41.2	54.3	63.6	69.7	65.0	58.3	49.2	39.3	27.8	43.3
1886	19.6	22.9	30.2	48.1	55.7	64.0	68.0	67.5	61.8	49.6	36.8	22.2	45.5
1887	20.2	23.2	26.3	41.1	62.5	65.7	75.6	66.5	57.7	47.0	37.6	27.6	45.9
1888	16.4	22.8	24.6	40.8	54.3	66.5	66.8	68.0	62.2	43.9	39.4	29.3	44.6
1889	29.1	18.1	33.9	45.1	58.4	65.3	70.2	66.0	60.5	44.0	40.3	35.2	47.2
1890	31.2	30.9	28.8	44.2	52.3	67.1	69.5	67.7	60.1	49.3	37.6	21.4	46.7
1891	25.9	28.3	30.8	45.3	52.0	66.4	66.4	68.5	66.2	48.3	38.4	35.5	47.7
1892	21.4	25.9	26.5	43.5	52.8	68.6	70.2	69.4	61.2	50.0	35.9	25.2	45.9
1893	15.5	20.6	29.5	41.1	54.1	68.2	69.8	68.8	58.0	52.0	38.2	27.5	45.3
1894	29.7	20.6	38.9	44.1	55.5	67.8	74.2	66.8	64.9	52.7	36.0	31.5	48.6
1895	21.8	16.9	26.9	44.4	59.0	......	......	71.2	61.7	45.4	39.6	31.4	....
1896	22.4	24.1	24.4	49.3	62.0	65.9	71.4	70.0	60.2	56.5	42.9	27.1	48.0
1897	23.2	26.1	33.8	45.0	55.4	62.3	73.6	67.6	62.3	52.6	39.7	29.2	47.6
1898	26.2	26.8	......	43.2	57.0	67.7	74.2	71.0	65.9	52.1	37.9	27.9	....
1899	22.1	20.4	30.4	46.6	57.6	69.5	71.2	71.6	60.6	53.5	38.9	30.0	47.7
1900	26.0	22.6	23.6	43.5	56.7	68.4	72.6	74.1	66.1	57.9	41.1	28.7	48.4
1901	26.1	18.5	32.2	46.5	56.9	68.9	76.6	71.0	64.0	51.4	34.3	27.7	47.9
1902	23.2	22.2	39.5	46.6	56.1	63.2	71.2	67.6	53.6	43.1	46.3	25.7	47.4
1903	25.7	28.1	42.2	45.9	60.4	63.2	70.8	65.5	64.4	52.5	36.2	23.3	48.2
1904	18.9	23.1	30.9	41.4	60.3	67.8	70.0	68.2	61.9	48.4	36.9	22.5	45.9
1905	19.8	18.9	33.1	44.8	57.5	66.4	71.8	68.7	63.7	52.4	37.6	32.0	47.2
1906	32.5	26.1	27.6	46.4	57.5	68.2	71.4	72.8	67.3	51.2	37.9	26.1	48.8
1907	24.9	19.5	38.1	40.2	51.3	64.0	71.2	68.4	64.4	47.9	38.7	31.8	46.7
1908	25.9	21.3	34.6	44.8	59.2	68.8	73.4	68.8	67.0	52.9	40.0	29.2	48.8
1909	27.7	28.6	31.0	44.3	57.9	67.2	69.6	70.0	63.5	47.7	44.5	25.7	48.1
1910	25.1	22.1	42.1	50.1	54.9	65.2	73.1	69.0	63.2	53.1	35.7	21.5	47.9
1911	24.9	26.6	30.9	44.8	64.9	67.5	74.4	70.9	62.8	50.7	36.6	35.1	49.4
1912	15.9	21.6	28.2	45.1	58.9	64.3	73.2	68.6	68.4	53.5	42.5	33.9	47.8
1913	32.7	18.8	36.9	48.3	56.9	66.8	72.1	70.0	61.3	54.0	44.5	33.6	49.7
1914	25.4	17.7	31.7	43.4	60.2	67.3	59.9	70.5	61.4	55.4	39.2	26.0	46.5
1915	26.5	29.6	31.0	52.3	52.6	64.6	69.6	67.8	65.6	52.6	41.1	28.5	48.5
1916	29.0	19.5	27.5	49.5	59.5	61.5	72.5	73.5	66.0	58.0	45.0	33.0	49.5
1917	24.6	20.2	33.8	45.8	49.5	63.9	71.8	70.5	60.1	45.9	35.3	19.5	45.0
Monthly averages	23.8	22.7	30.9	44.8	56.5	66.0	70.8	71.0	62.5	50.6	39.0	28.2	47.4

PRECIPITATION BY RAINFALL ONLY BY MONTHS SINCE 1882.

YEAR.	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Dec.	Total.
	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.	Inches.
1882	......	......	......	......	......	3.69	2.42	2.37	1.25	0.62	1.22	0.55	......
1883	0.48	1.44	0.88	1.58	4.45	4.12	2.98	3.47	2.12	2.10	1.54	0.73	25.89
1884	1.83	2.01	2.54	0.83	2.49	2.01	2.33	1.44	3.17	1.67	1.01	0.97	22.30
1885	1.07	0.61	0.12	1.26	1.58	2.49	4.64	5.02	2.11	2.88	1.36	0.76	23.90
1886	1.13	0.95	1.13	4.13	1.92	2.92	4.41	2.86	2.31	1.79	3.48	1.24	27.87
1887	0.18	2.97	0.48	1.37	0.46	2.01	6.37	3.03	0.75	1.74	1.58	1.35	22.29
1888	0.78	1.04	1.43	3.09	2.79	2.88	0.99+	4.02	2.73	3.47	2.02	1.24	20.48
1889	2.99+	0.25	0.66+	3.28	1.21	7.47	4.57	1.98	2.50	3.32	3.44	1.62	32.28
1890	2.16	1.45	2.16	2.20	5.49	4.26	1.07	4.34	5.81	4.54	2.40	......	36.88
1891	1.44	1.57	3.25	1.63	0.49	4.31	3.52	3.16	0.47	2.65	0.74	3.29	27.52
1892	0.57	0.88	0.55	0.67	4.04	3.95	1.89	4.77	1.12	1.34	1.67	0.72	23.17
1893	1.62	3.71	1.94	2.59	4.92	3.08	2.68	5.38	2.68	1.59	1.09	1.56	33.84
1894	2.21	2.71	1.36	2.43	7.03	1.77	1.50	1.22	4.64	3.59	0.43	0.47	29.36
1895	0.96	......	0.29	1.36	2.88	......	......	2.66	0.94	0.72	2.31	2.49	......
1896	1.19	2.28	0.84	0.41	2.31	3.71	4.12	3.33	4.27	2.26	2.18	0.71	27.61
1897	0.64	0.21	2.12	1.90	2.19	3.16	5.28	1.27	2.36	0.73	2.53	1.39	23.78
1898	1.74	0.33	1.54	2.03	1.90	2.37	1.32	3.60	1.86	3.83	2.03	0.33	22.90
1899	0.37	0.30	1.22	1.12	1.69	1.71	4.15	1.05	2.23	2.69	1.36	1.46	19.35
1900	1.43	2.42	0.02	0.95	1.71	1.45	6.53	1.75	0.91	3.65	6.13	0.78	27.73
1901	0.72	......	2.19	4.43	3.80	2.07	3.97	5.52	2.46	1.35	2.09	3.37	31.97
1902	0.86	0.66	1.94	1.92	2.84	4.33	5.29	2.41	2.88	2.32	0.74	0.74	26.89
1903	1.81	1.11	5.62	2.60	0.23	7.77	4.86	7.21	1.30	4.19	1.63	0.38	38.69
1904	0.80	1.03	2.41	1.67	4.04	3.37	5.73	2.56	3.26	2.06	0.26	1.42	28.61
1905	0.40	0.27	1.09	2.05	2.01	8.78	3.59	5.44	1.90	3.69	1.32	1.84	32.38
1906	1.46	0.53	1.60	2.08	4.24	5.31	2.37	3.68	2.16	3.56	1.40	1.54	29.93
1907	1.89	0.03	1.14	2.42	1.82	2.34	2.86	1.35	2.73	2.48	2.78	1.89	24.73
1908	0.68	1.12	1.24	3.28	3.57	1.96	4.72	1.79	1.66	2.73	0.88	0.43	24.06
1909	0.94	1.68	1.35	3.20	2.83	2.17	2.04	2.21	2.22	1.18	0.56	0.49	20.87
1910	0.87	0.53	0.28	4.56	3.45	1.55	2.39	5.47	3.29	1.73	0.62	0.38	25.12
1911	0.91	0.24	1.07	3.24	1.36	2.51	4.49	3.36	3.21	2.37	1.41	2.08	26.25
1912	0.20	0.95	1.92	3.41	7.27	2.09	4.85	2.21	5.89	1.42	1.48	1.13	32.82
1913	3.88	0.11	4.64	3.40	2.68	3.24	2.03	1.65	2.64	4.03	2.41	0.77	31.48
1914	0.98	0.15	1.28	3.10	4.00	2.69	2.18	6.05	1.62	1.55	1.08	1.13	25.81
1915	2.34	1.77	0.30	0.64	2.41	2.57	2.44	5.90	1.78	4.44	1.93	1.23	27.75
1916	2.69	1.05	0.50	3.40	5.69	5.83	1.78	3.47	2.15	1.41	1.83	1.49	31.29
1917	0.72	0.95	1.57	2.92	3.74	7.07	3.16	1.99	1.82	4.37	0.36	0.46	29.13
Averages	1.28	1.13	1.50	2.34	3.01	3.51	3.41	3.30	2.42	2.50	1.70	1.21	27.49

TOTAL PRECIPITATION: RAINFALL AND SNOW REDUCED TO EQUIVALENT RAINFALL, 1916–17.

YEAR.	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Dec.	Total.
1916	3.44	4.97	5.02	3.40	5.69	5.83	1.78	3.47	2.15	1.41	2.53	3.22	42.95
1917	1.97	1.90	2.77	2.92	3.74	7.07	3.16	1.99	1.82	4.37	0.71	3.03	35.45
Averages	2.70	3.43	3.89	3.16	4.71	6.45	2.47	2.73	1.98	2.89	1.62	3.12	39.20

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